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MEMORIAL COLLECTION

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Lewis

PROCEEDINGS

OF THE

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

ELEVENTH ANNUAL MEETING
New York
December 28–30, 1916

ABSTRACTS OF PAPERS
ABSTRACT OF EXECUTIVE PROCEEDINGS

Volume IV
No. 1

PROCEEDINGS OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS.

Eleventh Annual Meeting.

New York, N. Y., December 28–30, 1916.

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For the List of Members and the Constitution of the Society see the Register of the Federation of American Societies for Experimental Biology comprising the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, and the American Society for Experimental Pathology.

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THE DIASTATIC ACTIVITY OF THE BLOOD IN DIABETES.

BY VICTOR C. MYERS AND JOHN A. KILLIAN.

(From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York.)

Although it has been recognized since the time of Magendie that blood possesses active diastatic properties, no studies of a conclusive nature appear to have been published relative to the activity of the blood diastase in human subjects suffering from diabetes. This has probably been due to the lack of a sufficiently delicate and accurate method.

It has been found that the procedure introduced by Lewis and Benedict[1] for the estimation of the sugar of the blood, may be excellently utilized in the estimation of its diastatic activity. Two 2 cc. samples of oxalated blood are taken, one being employed as a control. The control tube is made up to 10 cc. with distilled water, and the tube to be employed for the test to 9 cc. Both tubes are now placed in a water bath at 40°C. As soon as the contents of the tubes have been brought to this temperature, 1 cc. of 1 per cent soluble starch is added to the second tube, the contents are mixed, and incubation is then carried out for exactly 15 minutes at 40°C.

After the incubation has been completed, about 1.0 gm. of dry picric acid is at once added to each tube and the mixtures are stirred. When the proteins are precipitated, the tubes are centrifuged and the supernatant fluid is filtered. The sugar in 3 cc. portions of the filtrates is now estimated according to the technique described by Myers and Bailey.[2] Correction is made for the sugar originally present in the blood (with the aid of the control) and for the slight reducing action of the soluble starch. The results have been recorded in terms of the percentage of the soluble starch (10 mg.) transformed to reducing sugars (calculated as glucose) by the 2 cc. of blood employed.

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[1] Lewis, R. C., and Benedict, S. R., *J. Biol. Chem.*, 1915, xx, 61.

[2] Myers, V. C., and Bailey, C. V., *J. Biol. Chem.*, 1916, xxiv, 147.

It is believed that 10 mg. of starch furnish a sufficient substrate for all ordinary conditions with human blood (except possibly in very severe diabetes), since practically identical results have been obtained with amounts of starch up to 40 mg. Glycogen offers no advantage over soluble starch, since the results are essentially identical. The possible error of glycolysis would appear to be a negligible one during a 15 minute period of incubation.

The diastatic activity of the blood, with this method, has been found to vary from 15 to 25 in a variety of miscellaneous conditions in the human subject. In cases of advanced nephritis with nitrogen retention and hyperglycemia, figures from 30 to 40 have been observed, while in eleven cases of diabetes the values ranged from 40 to 74. These results would suggest that the increased diastatic activity in both nephritis and diabetes (as shown by the analyses of the blood) may be the important factor in the production of the hyperglycemia in these conditions.

THE BLOOD LIPOIDS IN NEPHRITIS.

By W. R. BLOOR.

Lipemia has occasionally been reported in nephritis, and because of its importance in the study of fat metabolism an examination of the blood lipoids in a series of cases of severe nephritis was undertaken. The results showed mainly marked increases in the total fatty acids (due to fat) in the blood plasma and of lecithin in the corpuscles, with less marked and less frequent increases of fat and of cholesterol in the corpuscles. These abnormalities are essentially those found in the blood in alimentary lipemia and their presence in nephritis is believed to be due to a retarded fat assimilation which is one manifestation of a general phenomenon produced by the acidosis present in this condition.

THE LIPOIDS OF THE BLOOD IN RENAL CONDITIONS, WITH SPECIAL REFERENCE TO THE CHOLESTEROL CONTENT.

By ALBERT A. EPSTEIN AND MARCUS A. ROTHSCHILD.

By means of the newly developed methods for the examination of fats and lipoids in the blood, it has been possible for us to examine nearly 100 cases of renal disease and allied conditions.

The results may be briefly summarized as follows: In chronic parenchymatous nephritis (nephrosis), particularly in the edematous stage, the lipoids of the blood are exceedingly high; the cholesterol content, to which special attention has been given in this study, has been observed as high as 1.226 gm. per 100 cc. In other renal conditions, including surgical cases, no such increase has been encountered. Occasionally a moderate rise has been found, but as a rule this was associated with some other superimposed condition.

In uremic cases, particularly those showing high nitrogen retention in the blood, the lipoid content is very much diminished. The cholesterol in such cases may fall as low as 0.080 gm. per 100 cc. Several effusion fluids from cases of parenchymatous nephritis have been examined and only traces of lipoid have been found in them. The urines of these cases, when rich in protein material, show the presence of small amounts of lipoid.

The origin of the increase of the lipoid content of the blood in the parenchymatous form of nephritis is not clear. Undoubtedly a large portion, perhaps the greatest, is due to ingested lipoid material. A certain part may be ascribed to mobilization of body fat, particularly as these cases when recuperating show extreme emaciation. This high grade form of malnutrition is undoubtedly associated with considerable tissue degeneration, so that a certain portion of the lipoid in the blood must be attributed to that source. At any rate, the finding undoubtedly represents a condition of fat non-utilization, comparable to that found in diabetes and other nutritional disorders. This is evidenced also by the fact that when a diet consisting of large amounts of protein, a small amount of carbohydrate, and practically no fat, is administered for a long period of time, the excessive lipemia and lipoidemia gradually subside.

OBSERVATIONS ON ACID-BASE EQUILIBRIUM IN THE BODY.

By JOHN HOWLAND and W. McKIM MARRIOTT.

(From the Harriet Lane Home and Department of Pediatrics, Johns Hopkins University, Baltimore.)

Known amounts of hydrochloric acid, of acid sodium phosphate, and of sodium phosphate of blood reaction were administered to normal adults. Determinations on the urine showed that hydrochloric acid administration led to an increase in titratable acid (A) and a proportionate increase in ammonia excretion, the ratio A:NH_3 remaining constant. Acid sodium phosphate increased the titratable acid excretion but had no effect on ammonia excretion. The ratio A:NH_3 was greatly increased. Administration of phosphate mixtures of blood reaction ($P_H = 7.4$) led to a slight increase in the titratable acid and to a distinct diminution in ammonia excretion. The A:NH_3 ratio was considerably above normal limits.

These findings may be taken to explain, in part, at least, the low excretion of ammonia in the acidosis of nephritis, it having been previously demonstrated that this condition is accompanied by a retention of acid phosphate.

ADENINE AND GUANINE IN COWS' MILK.

By CARL VOEGTLIN and C. P. SHERWIN.

(From the Hygienic Laboratory, United States Public Health Service, Washington.)

From 100 liters of a mixed sample of cows' milk it was possible to isolate 500 mg. of adenine and 100 mg. of guanine by means of silver precipitation of the protein-free milk residue. These findings therefore prove the existence of two hitherto unknown constituents of milk.

THE ANABOLIC ACTION OF THE THYROID GLAND.

By N. W. JANNEY.

(From the Chemical Laboratory of the Montefiore Home and Hospital, New York.)

The catabolic action of the thyroid, as exemplified in the treatment of obesity, takes place when large doses of thyroid preparations are administered. It was studied by us in nitrogen partition experiments made on fasting dogs. A greatly increased excretion of all the nitrogenous urinary constituents, except creatinine, took place, indicating a stimulation of catabolic cellular metabolism. Fever and other toxic symptoms were likewise present. The anabolic action of the thyroid has remained obscure, as it can be demonstrated only by giving very small doses of the gland or its preparations. The results in many respects are the opposite of the catabolic and toxic action.

Using the nitrogen balance as a gauge of the effect of thyroid treatment, we have studied the results of administering small doses of thyroid preparations to cretins and exophthalmic goiter patients over extended periods. It could be shown that an added retention of nitrogen accompanied improvement in the clinical symptoms.

The same amounts of thyroid preparations fed to normal control persons on the same diet and under the same conditions were followed either by no change or by a reduction in the nitrogen balance.

In other preliminary experiments evidence has been obtained that the anabolic action which the thyroid gland exerts on protein metabolism is connected with its effect on carbohydrate metabolism. Thus the protein-sparing action of sugar which was found to be greatly reduced after thyroidectomy could be restored by feeding in addition small doses of thyroid.

ENZYME AND SUBSTRATE IN AUTOLYSIS.

By MAX MORSE.

The following reasons may be given for believing that the effect of acid introduced or already present in an autolyzing digest is upon the enzyme, and that the effect upon substrate, discovered by Bradley, is of secondary and not primary importance.

1. All known enzymes respond to alterations in hydrogen ion concentration. Therefore, if the effect of acid is upon the substrate from the inception of autolysis, as Bradley believes, no provision is made for this response and one must conclude that the enzyme is refractory to modification of the concentration of hydrogen ions. In this case the enzyme differs from all known ferments.

2. The substrate theory demands a high degree of specificity on the part of the enzyme, owing to the belief that the amount of substrate available for the enzyme is conditioned by the concentration of hydrogen ions. At the same time, Bradley has shown that the enzyme is not specific, since foreign proteins (casein, coagulated proteins, etc.) are digested.

3. The addition of coagulated proteins to a digest together with the addition of acid does not lead to acceleration of autolysis. This is interpreted on the substrate theory by assuming that there is no further activation of the enzyme. It is equally well interpreted as meaning that no more enzyme is introduced, for on introducing enzyme the rate is increased.

4. Inorganic colloids accelerate autolysis (Ascoli and Izar). Pincussohn, Wohlgemuth, and others have demonstrated an effect upon salivary amylase, pepsin, etc., when subjected to various inorganic suspensoids, so it is probable that a similar effect is exerted upon the autolytic enzyme.

5. Calcium salts, such as phosphate, sulfate, etc., show no acceleration in autolysis when used in hydrogen equivalent solutions, while similar salts of potassium, sodium, etc., show marked acceleration. The "salt effect" of Loeb is doubtless operative here and we have a basis for the suggestion herein made that the effect of acid is first to modify the permeability of the membranes and secondly to activate the enzyme.

ANTAGONISTIC ELECTROLYTES AND JELLY FORMATION.

By G. H. A. CLOWES.

When NaCl is added in increasing proportion to aqueous suspensions of soaps or lipoids, the dispersion of the particles in water is at first promoted, reaches an optimum at approximately 0.15 M NaCl, which corresponds approximately with the concentration of this substance in the blood, as noted by Koch. Subsequently an aggregating effect is exerted, precipitation occurring at a concentration of 0.35 to 0.4 M NaCl, which appears to be the limiting concentration for the maintenance of life of marine organisms, of mice on intravenous injection, and the precipitation point for emulsions, jellies, and a variety of other physical systems.

Koch's curve of the amount of $CaCl_2$ required to precipitate lecithin at different concentrations of NaCl corresponds closely with curves obtained by the drop method[3] when NaCl is added in increasing proportion to an aqueous phase containing a constant amount of soap passed through neutral oil. The initial rise in drop number attributable to preferential adsorption of Cl ions promoting dispersion of the soap particles reaches an optimum at 0.15 to 0.25 M and subsequently diminishes, presumably owing to the relatively increased adsorption of the Na ion with rise in concentration. Soap jellies may be obtained at concentrations between 0.25 and 0.4 M NaCl provided sufficient NaOH was added to the original system to insure a sufficiently high initial dispersion of the soap particles (as indicated by the optimum drop number), to prevent aggregation of the particles and their deposition under the influence of gravity. If Brownian movement is maintained up to a certain concentration of NaCl, subsequent adsorption of Na ions causing reduction of negative charge and change of surface tension results in such a distortion of the individual particles that they coalesce simultaneously with one another to form a jelly-like structure containing water in the interstices.

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[3] Clowes, G. H. A., *J. Physic. Chem.*, 1916, xx, 407.

The close similarity in the antagonistic curves of $CaCl_2$ and NaCl and other salts having a more readily adsorbed anion in such widely diversified systems as living protoplasm, the blood clot emulsions, soap jellies and films, and soap and lipoid suspensions, and the common limiting concentrations at which individual salts like NaCl and $CaCl_2$ produce inhibiting effects in all these systems, concentrations which differ entirely from those at which similar effects may be observed on proteins, suggest the probability that protoplasmic equilibrium and the formation of reversible protoplasmic jellies are dependent upon what may be designated as an imperfect reversal of phase relations promoted by the action of antagonistic electrolytes on interfacial films of soap and lipoid just as in reversal of equilibrium in the case of emulsions of oil and water.

ELECTROLYTES AND ANAPHYLAXIS.

By G. H. A. CLOWES.

Ca salts appear to counteract sensitization phenomena by diminishing the permeability of the tissues to water. The fact that Ca salts, owing to their aggregating effect on soap films, may be used to counteract increased permeability resulting from adsorption or interaction of negative ions, and that the ratios in which various salts having readily adsorbed anions are antagonized by salts of Ca are approximately the same in such widely diversified systems as marine organisms, living mice, blood coagulation, complement hemolysis, soap and lipoid suspensions, and drop systems,[3] coupled with the drop in blood coagulation in anaphylaxis, suggests the probability that the antigen-antibody combination affords a link between water and some non-aqueous phase in the protoplasmic film whereby the latter is subjected to the action of readily adsorbed anions already present in the system with a resulting sudden increase in permeability beyond those limits within which normal protoplasmic function may be maintained.

Support for this contention may be found in the well known surface tension effects and the rôle played by electrolytes in immune phenomena; and a practical analogy is to be found in experiments carried out by means of the drop method, demonstrating that water containing NaOH or NaOH + NaCl may be passed through neutral oil without causing any change in the drop number, and water or water + NaCl may be passed through oil containing fatty acid without change, but when NaOH and fatty acid are both present in the system either as soap in the water phase or as NaOH in the water phase and fatty acid in the oil phase, a considerable rise in the drop number occurs, and if NaCl is present there is in addition a still further rise.

With a drop system in which the conditions of equilibrium appear to be comparable with those of living protoplasms, a rise from ten to fifty drops may be effected by introducing a given amount of soap into the water phase or NaOH into the water phase and fatty acid into the oil phase, and a further rise to 300 drops may be effected by using physiologically normal salt solution instead of water. The great increase in permeability indicated, far in excess of that required to cause destruction of protoplasm, could only be effected in the presence of both alkali and fatty acid, presumably owing to the affinity of water for NaOH, of NaOH for fatty acid, and fatty acid for oil; and the consequent establishment of chemical or physical contact between the water and oil phases without which the dispersing effect of the readily adsorbed Cl ion could not come into play.

CALCIUM AND PHOSPHORUS IN THE BLOOD OF LACTATING COWS.

By EDWARD B. MEIGS and N. R. BLATHERWICK.

(From the Bureau of Animal Industry, United States Department of Agriculture, Washington.)

Calcium, nitrogen, total phosphorus, and lipoid and inorganic phosphorus have been determined in the blood and plasma of cows and heifers of various ages, on various rations, and at various stages of pregnancy and lactation. The results are still highly incomplete, but are sufficient to indicate certain relations.

The calcium content of cows' plasma has been found to be surprisingly constant. It is somewhat over 0.011 per cent in very young heifer calves, and falls off gradually with increasing age until it reaches about 0.010 per cent toward the end of the first year, from which point it remains nearly constant. Our results indicate that it is not appreciably altered by pregnancy, lactation, or by considerable changes in diet. Our highest figure for plasma calcium has been 0.0114 per cent, and our lowest 0.0086 per cent.

The total, lipoid, and inorganic phosphorus contents of normal plasma are all extremely variable. In our experiments the total phosphorus has varied from 0.0085 per cent to 0.0178 per cent; the lipoid phosphorus, from a little under 0.002 per cent to a little over 0.008 per cent; and the inorganic phosphorus, from about 0.004 per cent to about 0.008 per cent. The lipoid phosphorus has been found lowest in very young calves, highest in cows at about the 6th month of lactation, and intermediate in farrow heifers from 1 to 2 years old, and in pregnant cows at the end of the previous lactation period. The inorganic phosphorus has been found highest in young calves, and is likely to be low in mature cows just after calving. It seems to depend to some extent on the amount of grain fed.

In numerous samples of plasma, the total, lipoid, and inorganic phosphorus contents have been independently determined, and it has always been found that the sum of the lipoid and inorganic fractions is equal, within the limits of experimental error, to the total.

Our nitrogen results indicate an increase in the ratio, protein:

water, in plasma with increasing age, and some individual variation in this ratio; but little change as the result of pregnancy, lactation, or change in rations.

STUDIES IN THE MECHANISM OF ABSORPTION FROM THE COLON

By SAMUEL GOLDSCHMIDT and A. B. DAYTON.

(From the Hunterian Laboratory of Experimental Pathology, Johns Hopkins Medical School, Baltimore.)

Sodium chloride solutions of concentrations above 1.2 per cent when placed into the colon of the dog cause a passage of water into the colon contents. At the same time chlorides are passing into the blood. There is an immediate drop in the concentration of chlorides. The Δ of the colon contents shows an immediate decrease of osmotic pressure. The concentration of chlorides and the Δ at the point where the volume ceases to increase is above the concentration of chlorides and the Δ of the blood.

The concentration of chlorides finally comes to an equilibrium with the chlorides of the blood. An increase of chlorides in the blood causes a readjustment of this equilibrium.

The Δ of the colon contents is not accounted for by the chlorides; hence "other substances" have diffused into the colon from the blood.

Chlorides may be withdrawn from the blood until the concentration in the colon reaches 0.12 to 0.17 per cent, when there is a reabsorption of the chlorides; nevertheless the concentration of chlorides in the colon increases to blood level. The colon is therefore not an "irreciprocal membrane." It is essentially a permeable membrane towards sodium chloride solutions.

A MODIFICATION OF FOLIN'S METHOD FOR THE ESTIMATION OF CREATININE IN BLOOD.*

By THEODORE KUTTNER.

(From Mt. Sinai Hospital, New York.)

The modified method suggested reduces the disturbing factor of the yellow color of the picric acid, because of the smaller amount of this substance used than in the original Folin method.

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*Read by invitation.

For similar amounts of blood Folin's method requires thirty-three times as much picric acid as in the new procedure which depends upon removal of the proteins before the addition of the picric acid. Details of the procedure will be published shortly.

A QUICK TITRATION METHOD FOR DETERMINING SMALL AMOUNTS OF URIC ACID.

By J. LUCIEN MORRIS.

(From the Laboratory of Biological Chemistry, Washington University, St. Louis.)

Precipitation of uric acid as zinc urate is used as the basis of separation of uric acid from other substances in body fluids which would interfere with a subsequent permanganate oxidation. The method is described in detail for urine but has application as well to blood. Albumin is removed by treatment either with trichloroacetic acid or *m*-phosphoric acid. An aliquot part of albumin-free filtrate is added to a special solution of sodium lactate, sodium acetate, and acetic acid, and the phosphates and ammonia are removed by precipitation at room temperature with uranium acetate. The solution obtained after filtering or centrifuging will allow its uric acid content to be removed completely as zinc urate upon the successive addition of zinc acetate and sodium carbonate. Centrifuging for a minute separates the precipitate and the liquid may be poured off. Solution of the precipitate is obtained by the addition of acetic acid and a reprecipitation with sodium carbonate assures the absence of substances which might interfere with the permanganate titration. Solution of the precipitate in acetic acid, heating to 80°C., and adding phosphoric acid until zinc phosphate dissolves are the conditions which immediately precede the titration with 0.005 N potassium permanganate. Use of sulfuric acid gives a figure about 10 per cent higher, due probably to purines which are precipitated with zinc but which are not oxidized with permanganate in acetic acid solution.

NOTE ON A TITRATION METHOD FOR DETERMINING MINUTE QUANTITIES OF ACETONE.*

By ROGER S. HUBBARD.

(From Washington University, St. Louis.)

The solution of iodine used in the regular Messinger determination of acetone is diluted in 3 per cent potassium iodide, and used for the determination of minute quantities of acetone. 15 gm. of potassium iodide are dissolved in water in a 500 cc. volumetric flask, appropriate amounts of the stock iodine solution added, and made to volume. From the stock solution of approximately 0.1 N concentration (1 cc. equals 1 mg. of acetone) 0.01 N and 0.002 N solutions are so prepared. Thiosulfate solutions of corresponding strength are easily prepared, and are fairly stable.

In carrying out the determination the acetone solution is distilled to a volume of 250 cc. and 50 cc. are taken for a determination. 40 cc. of 0.01 N iodine solution are added, followed by 1 cc. of 10 × N NaOH. The solution is shaken, and allowed to stand 10 minutes. It is then acidified with H_2SO_4, and the excess of iodine titrated with 0.01 N thiosulfate solution, using 1 to 2 cc. of 0.25 per cent starch as indicator.

If the amount of acetone is less than 0.2 mg., the procedure is repeated, using 10 or 20 cc. of 0.002 N iodine solution, and titrating with 0.001 N thiosulfate. A blank should be run on the NaOH used. The iodine solution is permanent for 4 or 5 days, and thiosulfate solution for 48 hours. Amounts of acetone as small as 0.01 mg. may be accurately determined by this method

THE ALLEGED NINHYDRIN REACTION WITH GLYCEROL, ETC.

By VICTOR JOHN HARDING.

(From the Biochemical Laboratory, McGill University, Montreal.)

The author found that in following the work of Halle, Loewenstein, and Přibřam on the production of a ninhydrin reaction by glycerol, specimens of glycerol of different origin gave different results. The production of a positive ninhydrin reaction with glycerol is believed to be due to the presence of nitrogenous impurities.

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*Read by invitation.

THE USE OF HEXAMETHYLENETETRAMINE AS A RENAL FUNCTIONAL TEST, AND ITS COMPARISON WITH OTHER FUNCTIONAL TESTS.*

By W. G. LYLE.

(From the Harriman Research Laboratory, Roosevelt Hospital, New York.)

A method for the quantitative determination of urotropin in the urine has been described by Falk and Sugiura.[4] They found that if 15 grains of urotropin were given to a person with normal kidney function, and the urine was collected for 12 hours, they were able to recover from 40 to 80 per cent of the urotropin unchanged; on the other hand, if the patient was suffering from nephritis, little or no urotropin was excreted. It was suggested that this fact should be utilized as a test for the functional activity of the kidney. A comparison of this method with other functional kidney tests was undertaken.

In general it may be stated that in cases where the clinical symptoms point undoubtedly to nephritis nearly all the functional tests agree fairly well. In early cases, however, where the diagnosis is in doubt, urotropin is of value. The failure of the kidney to excrete the urotropin molecule is a very early symptom of interference with its function. It is of interest to note that this phenomenon coincides closely with the finding of an increased amount of uric acid in the blood, and with the changes in the kidney function, as shown by the Mosenthal test meal, especially those cases showing a marked increase in the volume of night urine. In this type of case we have frequently found a good, or even high dye test and a normal non-protein nitrogen and urea. Whether some of these cases will develop a true nephritis remains to be seen, but that there is present some disturbance of kidney function is evident.

In conclusion, from our work with these tests, one fact stands out clearly,—that it is unwise to base a diagnosis on the result of one or two tests as there are cases in which the uric acid in the blood is normal while the urotropin, Mosenthal meal, and the dye test show kidney disturbance. On the other hand, cases of gout may show a high uric acid content in the blood, while the other tests are normal. The advantage of urotropin is that the test is easily carried out and gives the patient no inconvenience. We therefore recommend that in a suspected case the patient should be given 15 grains of urotropin, dissolved in a glass of water, shortly before the evening meal, at which time fluid should be taken to the amount equivalent to two more glasses of water; a 12 hour specimen should be collected and examined for urotropin; if none is found, or a small amount, the other tests should be carried out.

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*Read by invitation.

[4] Falk, K. G., and Sugiura, K., *J. Pharm. and Exp. Ther.*, 1917, ix, 241.

THE CHOLESTEROL CONTENT OF THE BLOOD IN VARIOUS HEPATIC CONDITIONS.*

By M. A. ROTHSCHILD and J. FELSEN.

(From the Department of Physiological Chemistry, Pathological Laboratory, Mount Sinai Hospital, New York.)

In obstructive icterus the cholesterol content of the blood is markedly elevated and bears a definite relationship to the intensity of the icterus. In conditions such as cirrhosis, acute yellow atrophy, pernicious vomiting of pregnancy, or extensive carcinomas of the liver, the cholesterol content of the blood is not increased and is usually reduced. The cholesterolemia is thus not proportionate to the intensity of the icterus. In other forms of icterus, as seen in Banti's disease, Gaucher's disease, splenomegaly with icterus, and pernicious anemia and other blood diseases, there is no increase in the blood cholesterol, and in most of these conditions the amount present is decidedly less than normal.

THE USE OF PANCREATIC VITAMINE IN CASES OF MALNUTRITION.

By WALTER H. EDDY.

(From the Department of Pathology of the New York Hospital.)

The report covers the preliminary study of the use of pancreatic vitamine in cases of marasmus. Up to the present time the treatment has been administered to eleven cases, seven of which form the basis of the present paper; the other four are not sufficiently advanced to supply data at this time.

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* Read by invitation.

The treatment consists in feeding children 2 gm. per day of Lloyd's reagent which has been activated by pancreatic vitamine after the method of Seidell. Previous work had demonstrated that 50 gm. of Lloyd's reagent shaken with 1 liter of the water-soluble portion of an alcohol extract of pancreas remove from that volume its growth-stimulating power. In these experiments 1 gm. of Lloyd powder contains the vitamine from 54 gm. of pancreas. (This assumes that the removal of the vitamine is quantitative.) The powder is administered by mixing 1 gm. with a feeding of cereal. No difficulties have arisen in getting the children to take this material. The success of the experiment is judged by noting whether growth develops after administration of the vitamine, no other change being made in the diet.

The results suggest at least the possibility of stimulating assimilation by the artificial feeding of pancreatic vitamine. The dosage and the conditions favorable and unfavorable to its effectiveness are under investigation.

THE URIC ACID CONTENT OF THE BLOOD OF THE NEW-BORN.

By F. B. KINGSBURY, J. P. SEDGWICK, and L. J. ROBERTS.

Uric acid determinations in the blood of the new-born showed that the concentration of uric acid varied from 1.8 to 3.5 mg. per 100 gm. of placental blood, and from 2.7 to 4.9 mg. per 100 gm. of blood drawn from the new-born 8 to 120 hours later. The uric acid content in the case of a new-born 8 hours old was 4.9 mg. per 100 gm. of blood. At the end of 10 days this value had dropped to 1.4 mg. In the case of another baby the placental blood contained 1.9 mg. which rose to 4.0 mg. 46 hours later, and at the end of 9 days was 3.8 mg. Both placental blood and blood taken a few hours after birth contain more uric acid [illegible] of the normal adult. A definite increase after birth is shown and this occurs during the same period of time that uric acid infarcts in the kidney are known to be formed.

THE PARTITION OF NON-PROTEIN NITROGEN IN THE BLOOD OF FRESH WATER FISH.

By D. WRIGHT WILSON and EDWARD F. ADOLPH.

(*From the Laboratory of Physiological Chemistry, Johns Hopkins University, Baltimore, and the United States Fisheries Biological Station, Fairport, Iowa.*)

The whole blood and plasma from several species of fresh water fish were analyzed for non-protein nitrogen, urea, ammonia, amino nitrogen, uric acid, creatinine, and creatine. The fish studied were the sturgeon, gar, carp, croppie, catfish, and sheepshead. The total non-protein N was lower in the ganoids (29 to 31 mg. per 100) than in the teleosts (42 to 59 mg.). The plasma contained less than the whole blood in all cases. The urea nitrogen was unusually low in most specimens, varying from 1.1 to 16 mg. per 100 cc. of whole blood and from 0.4 to 13 mg. per 100 cc. of plasma. The urea content of the plasma was less than whole blood in all cases. The amino nitrogen fraction made up from 60 to 81 per cent of the total non-protein nitrogen of the whole blood, and from 33 to 45 per cent of the total non-protein nitrogen of the plasma. The ammonia, uric acid, and creatinine contents of whole blood were similar to the other values reported for fish. The creatine content of plasma was higher than that of the corpuscles. The considerable variations in the composition of the blood of fish and higher animals suggest differences in metabolism.

THE DISTRIBUTION OF CREATININE AND CREATINE BETWEEN THE CORPUSCLES AND PLASMA OF THE BLOOD.

By ANDREW HUNTER and WALTER R. CAMPBELL.

(*From the Department of Pathological Chemistry, University of Toronto.*)

In forty-eight specimens, two of which came from rabbits and the remainder from human subjects, the creatinine contents of the whole blood and of the plasma were separately determined. Creatine estimations were similarly carried out in forty-two cases, in thirty-three of which the measurement of corpuscular volume enabled us to calculate the concentration of both substances in the corpuscles also. These data were supplemented in twenty-six cases by a determination of total and preformed creatinine in the urine excreted at the time of the observation.

The single generalization which we can draw in the meantime from our results is that the concentration of both creatine and creatinine is considerably lower in plasma than in whole blood, so that, bulk for bulk, the corpuscles are much richer in these substances than the plasma. The *plasma* creatinine of normal individuals was always less than 1 mg. per 100 cc.; in all but two it fell between 0.72 and 0.92, the average being 0.82. The plasma creatine in the same subjects ranged from 0.94 to 3.5; but in two-thirds of them it lay between 1.4 and 1.7, and the average for human individuals was 1.6. The creatine and creatinine content of the *corpuscles* varied within rather wide limits, but the second was always present in much greater quantity than the first.

When creatinine is retained, as in the nephritic cases, it appears generally to accumulate in the corpuscles as well as the plasma. We have, nevertheless, encountered some cases in which the plasma creatinine seemed to have increased out of proportion to that of the whole blood, and others in which blood containing an abnormally large quantity of creatinine yielded a plasma with the normal amount. These occasional findings encourage us to hope that the plasma creatinine, as distinct from the blood creatinine, may prove to have a diagnostic value of its own.

The concentration of creatine in the plasma does not appear to bear any relation to the occurrence of creatine in the urine. We have obtained no indication of the existence of a threshold for creatine.

Observations on parturient women seem to indicate that there is a ready interchange of both substances between the mother and the fetus. The concentration of each is nearly the same in both bloods, and especially in the plasmas. Where a difference exists it is almost always in the direction of a somewhat higher concentration in the placental blood. In the majority of our cases the creatinine content of maternal and placental blood was above the normal.

The results reported are of a preliminary character only, and the work is being continued along each of the lines suggested.

THE EFFECT OF ARTIFICIAL RESPIRATION ON GLYCEMIA.

By I. S. KLEINER and S. J. MELTZER.

(From the Department of Physiology and Pharmacology of The Rockefeller Institute for Medical Research.)

The hyperglycemia which is produced by an intravenous injection of magnesium sulfate was shown in a previous report[5] not to be due to asphyxia, as had been suggested.[6] In those experiments artificial respiration had been given in order to prevent asphyxia, and, nevertheless, a marked hyperglycemia had been caused by the magnesium injection. It occurred to us later that possibly these results might have been due simply to the artificial respiration, since Henderson and Underhill[7] have reported that acapnia may produce an increase in the percentage of blood sugar. We have therefore determined the influence of artificial respiration upon the blood sugar concentration.

Ten dogs were used in this work. Under local anesthesia a cannula was inserted in a femoral artery and a tracheal cannula introduced for artificial respiration. In five experiments there were 20 or 21 interruptions of the air current per minute, and in five 40 to 44 per minute. Artificial respiration of the ordinary type, but with comparatively high air pressure, was administered for 1 hour. In none of the experiments was there any marked increase in the blood sugar, 0.04 per cent being the greatest increase in any. In five out of the ten experiments there was no increase whatever.

It is thus evident that vigorous artificial respiration for 1 hour causes no appreciable effect upon the percentage of sugar in the blood and therefore the hyperglycemia present when $MgSO_4$ is injected cannot be the result of an overventilation by the artificial respiration.

THE NATURE OF THE BLOOD SUGAR.

By HUGH McGUIGAN.

(From Northwestern University Medical School, Chicago.)

The work is based mainly on the known fact that picric acid does not interfere with the determination of blood sugar by

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[5] Kleiner, I. S., and Meltzer, S. J., *J. Biol. Chem.*, 1916, xxiv, p. xx.

[6] Underhill, F. P., and Closson, O. E., *Am. J. Physiol.*, 1906, xv, 321.

[7] Henderson, Y., and Underhill, F. P., *Am. J. Physiol.*, 1911, xxviii, 275.

Fehling's solution. The results, however, were corroborated by other methods. If the filtrate from the picric acid is analyzed directly it is found to contain on the average about 25 per cent of the total blood sugar. When, however, the picric acid filtrate is boiled with 4 per cent acetic acid for 2 minutes the sugar yielded rises on the average four times. The results indicate a hydrolysis probably of a dextrin. The dialyzed serum gives the same results. Ether anesthesia changes the ratio of the polysaccharide (?) to the dextrose from 4:1 approximately to 1:1. In severe diabetes the ratio is approximately 1:1.

There is a possibility of an interfering substance in the picric acid filtrate which may hold the cuprous oxide in solution, but we have been unable to find such a body. Consequently we think that any one method of blood sugar analysis is insufficient, that the ratio of the free to the combined or polysaccharide should be determined, and that the ratio of the two is of significance in diabetes. The polysaccharide or combined form corresponds to what has been called virtual sugar.

THE ENZYMES OF THE TUBERCLE BACILLUS.

By H. J. CORPER and H. C. SWEANY.

(From the Municipal Tuberculosis Sanitarium of the City of Chicago.)

Tubercle bacilli both of the human and bovine variety possess autolytic enzymes, as indicated by the liberation at incubator temperature of non-coagulable nitrogen, and of amino-acid α-nitrogen after the bacilli have been killed by toluene and chloroform. The bacilli themselves, or autolysates therefrom, also possess a trypsin-like enzyme capable of splitting proteins in alkaline solution, a weak pepsin-like enzyme capable of splitting proteins in acid, an erepsin-like enzyme capable of decomposing peptone in acid solution, a nuclease capable of acting upon nucleic acid, and a urease capable of decomposing urea. The tubercle bacilli, or autolysates therefrom, do not possess enzymes capable of hydrolyzing starch or inverting saccharose at incubator temperature, at least not to an extent capable of demonstration by the delicate Lewis and Benedict colorimetric picramic acid method. Autolysates from tubercle bacilli do not possess enzymes capable of digesting elastic tissue prepared from lamb's lung, or connective tissue prepared from tubercles, at least not by the methods used for demonstrating these enzymes. The demonstration of the latter enzymes of necessity had to be by indirect methods of attack and are, therefore, open to criticism.

A STUDY OF FOOD FISHES. THE COMPLETE ANALYSIS OF TWENTY COMMON FOOD FISHES, WITH ESPECIAL REFERENCE TO A SEASONAL VARIATION IN COMPOSITION.

By E. D. CLARK and L. H. ALMY.

(From the Food Research Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.)

In the present investigation specimens of twenty different species of fish were analyzed, some only once and others twice or more times during the year. In general, there is little variation in the amount of nitrogenous and ash constituents, but fat is the great variable, its amount depending upon the season, age of fish, and relation to time of spawning and feeding. The typical migratory fish, like shad, bluefish, weakfish, and mackerel, sometimes show variations from less than 0.5 per cent to over 16 per cent, depending upon the factors mentioned above. On the other hand, typical bottom fish, like cod, haddock, and flounders, have a minimum amount of fat which varies but slightly compared with surface and migratory species. Analysis of individuals taken from the same school of fish also show variations in fat content from 1 to 8 per cent in the case of weakfish. Some species, like shad and salmon, are fattest in the spring, while others, like bluefish and butterfish, are at their best in the fall just after the active feeding period. In the case of shad, the percentage of fat decreased from a maximum of over 14 per cent in April before spawning to a minimum of less than 3 per cent in June when the fish were spent after spawning.

In the case of the nitrogenous constituents of fish there is much less variation than in the fat. The coagulable and hot-water-soluble constituents (gelatin-like substances) vary somewhat with the species but change little from spring to autumn or winter. There were striking differences in the percentages of ammonia nitrogen, but it is not certain whether they were due to individual or species differences or to the varying leaching effect of water from melting ice in which the fish were packed for shipment.

A NOTE ON CERTAIN CHEMICAL CHANGES IN SHUCKED OYSTERS UNDER REFRIGERATION, AND METHODS OF DETECTING THESE CHANGES.

By E. D. CLARK and L. H. ALMY.

(*From the Food Research Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.*)

The work reported deals with chemical methods of supplementing bacterial counts in the analysis of oysters. Preliminary experiments showed that freshly shucked oysters standing at temperatures above the freezing point yielded regularly increasing percentages of ammoniacal nitrogen by aeration, amino-acid nitrogen, and reducing substances.

Larger scale studies were then made upon lots of oysters held at ice box temperatures (45° F.) and in cold rooms at 40° F. and 34° F., respectively. All these temperatures are regularly employed in the commercial handling and transportation of oysters, either in car lots or small packages. To simulate commercial practices in handling and transporting oysters, samples were held at different temperatures for various periods of time up to 15 days. During storage the ammoniacal nitrogen showed the most marked increases, but the low temperature of 34° F. had a great inhibiting effect on the production of ammonia. The amino-acid nitrogen and reducing substance (probably sugar) did not show increases of the same magnitude as ammonia because they may have been assimilated by bacteria, while ammonia is an end-product of bacterial metabolism. The amino-acid nitrogen was determined by Van Slyke's gasometric method and by Sörensen's formol titration method, both of which procedures gave satisfactory and concordant results on oyster extracts. The reducing substances were estimated by Benedict's quantitative method for sugar, using alkaline copper solution.

In general, the lower temperatures greatly retarded the formation of ammonia during storage, but apparently did not affect the other changes to as great an extent, possibly because increases in amino-acid nitrogen and reducing substances were due to enzymes which were much less retarded by low temperatures than was the growth of bacteria concerned in the production of ammonia. The reducing substance may have been glucose arising from the hydrolysis of glycogen in the oyster tissues. Living oysters, or those suddenly killed by plunging into boiling water, contained hardly a trace of reducing substance. As is the case with all perishable substances, decomposition in oysters is not prevented by low temperatures but is merely retarded, especially at temperatures near the freezing point.

PRELIMINARY OBSERVATIONS ON THE REGULATION OF THE CALCIUM EXCRETION IN THE DOG.

By MAURICE H. GIVENS.

(From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.)

A dog weighing 13.3 kilos has been maintained in nitrogenous equilibrium over a period of 60 days, during which time the calcium balance has been negative. This was brought about by feeding on a meat, lard, cracker meal diet. When for the cracker meal dried skimmed milk and sucrose were substituted, on the basis of nitrogen and calorie equivalent, there was an approach toward a calcium balance. The dried skimmed milk diet contained six and a half times as much calcium as the meat, cracker meal ration.

When sodium bicarbonate (0.5 gm. per kilo) was added to the meat, cracker diet, no effect was produced on the calcium output. No effect was observed on the addition of bicarbonate to the dried milk feed.

On the other hand, when hydrochloric acid (0.11 gm. per kilo) was added to the meat, cracker diet, there was a prompt increase in the excretion of calcium by way of the kidneys, presumably through diversion from the intestinal path. When the acid was superimposed upon the high calcium diet there was likewise an increased urinary excretion of calcium though not at the expense of the fecal output.

The reported effect of alkali bicarbonate upon the calcium output during various levels of calcium intake has been confirmed on a second dog. These studies are being continued.

THE INFLUENCE OF ACID AND ALKALI ON THE ABSORPTION OF GLUCOSE FROM THE INTESTINE.

By JOHN R. MURLIN.

(*From the Physiological Laboratory of Cornell University Medical College, New York City.*)

Loops of the small intestine of chloretonized dogs were measured off so as to be as nearly equal in length as possible, washed out with warm Ringer's or Tyrode's solution, and then filled with equal quantities of glucose solutions, one having a certain strength of alkali measured as anhydrous sodium carbonate, and the other an equivalent amount of hydrochloric acid. The solutions were permitted to remain in the intestine for from 30 to 165 minutes, then withdrawn, and titrated for sugar with Benedict's solution. Out of nearly two dozen experiments only two failed to show more absorption from the alkaline medium than from the acid, and in one of these there was sign of leakage through the ligatures.

The following may be cited as examples.

Dog No.	Glucose given.	Strength of alkali.	Absorption per minute.		
			Alkaline loop.	Acid loop.	Length of time.
	gm.		*mg.*	*mg.*	*min.*
II	2.97	0.3 per cent.	20	10	34 and 37
III	2.94	0.5 " "	20	12	120
IV	2.14	0.3 " "	13	10	165
VI	1.90	0.3 " " in both loops.	14	15	90
A	2 per cent.	0.1 N	0.36	0.63	30
B	2 " "	0.2 "	0.035*	0.166*	45

* Per cm. length. The loops were not equal in length.

In general, the greater the concentration of the acid and alkali, the greater was the difference in absorption between the two loops. In several instances the mucous lining of the alkaline loop was ground and hydrolyzed with hydrochloric acid, but no reducing substance was found.

The explanation of the difference seems to be the different effects of alkali and acid on protoplasm, the one tending to increase permeability, the other to decrease it.[8]

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[8] Osterhout, W. J. V., *J. Biol. Chem.*, 1914, xix, 335.

AN EXPERIMENTAL STUDY OF LIPOLYTIC ACTIONS.

By K. GEORGE FALK.

(From the Harriman Research Laboratory, Roosevelt Hospital, New York.)

The inactivation of esterase and lipase from castor beans by acids, bases, neutral salts, alcohols, acetone, esters, and heat led to the hypothesis that the lipolytically active grouping is the tautomeric enol-lactim form of the peptide linking which becomes inactive on rearrangement to the keto form. Experiments testing this view resulted in the production of lipolytically active substances by the action of alkali on castor bean globulin, casein, and gelatin. Further confirming evidence was obtained on studying the ester-hydrolyzing action of glycine, glycyl-glycine, and hippuric acid at different hydrogen ion concentrations. Summarizing, it may be said that, given a definite chemical grouping the nature of which has been indicated, and which may be present in different classes of substances, certain definite lipolytic actions will result.

A METHOD FOR THE SEPARATION OF THE DIETARY ESSENTIAL, "FAT-SOLUBLE A," FROM BUTTER FAT.

By E. V. McCOLLUM, N. SIMMONDS, and H. STEENBOCK.

(From the Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.)

Fat-free milk, when included in a diet consisting otherwise of purified food substances, promotes growth and prevents decline of animals in a manner which indicates that it still contains the fat-soluble essential in appreciable amount. This led us to suspect that it is appreciably soluble in water. Experiments have been conducted in which butter fat was employed which had been melted and thoroughly agitated with twenty successive portions of water. After this treatment it is no longer effective in inducing growth when fed with suitably constituted diets. Change to unwashed butter fat was followed by prompt resumption of growth.

ANTINEURITIC SUBSTANCES FROM EGG YOLK.

By H. STEENBOCK.

(From the Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.)

By means of neutral solvents there was prepared a water-acetone-soluble fraction from egg yolk which in small doses by intraperitoneal injections was able to cure a pigeon suffering from polyneuritis. The antineuritic principle was found stable to concentrated hydrochloric acid at 98° and to concentrated alkalies at room temperature, but readily destroyed by dilute alkalies at the boiling temperature. Phosphotungstic acid precipitated it incompletely. It was not adenine.

THE ISOLATION OF STACHYDRIN FROM ALFALFA HAY.

By H. STEENBOCK.

(From the Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.)

l-Stachydrin has been isolated in pure form and as the hydrochloride from the phosphotungstic acid fraction of the water-soluble constituents of alfalfa hay. For its identification the picrate, chloroplatinate, aurate, methyl ester, and methyl ester chloroaurate were prepared and found characteristic. The optical activity was found slightly below the reported value though of the same sign. Both the Kossel and the Van Slyke methods when applied directly to alfalfa hay nitrogen give erroneous values for the diamino-acids, the error in the first case falling upon the lysine fraction and in the second case upon the histidine fraction.

A METHOD FOR THE IDENTIFICATION OF PHENYLALANINE-URAMINO-ACID IN THE PRESENCE OF UREA AND AMINO-ACID.*

By ALICE ROHDE.

So far it is undetermined whether uramino-acids exist preformed in the body fluids or whether when isolated they are the products of the interaction of urea and of amino-acid present.

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* Read by invitation.

Large intravenous doses of phenylalanine were administered to cats under urethane anesthesia, the urine was collected from bladder cannulas, and the urea completely destroyed as secreted by urease. After extraction of the urea-free material with acetic ether the aqueous residues left on distillation with steam were analyzed for traces of uramino-acid by the gasometric method for the determination of amino nitrogen. No phenylalanine-uramino-acid could be detected. The determination of phenylalanine-uramino-acid added to urine and Jack bean extract is approximately quantitative by this method, so that one may expect to identify minute amounts of preformed uramino-acid if present.

THE ABSORPTION OF ALCOHOL AND ITS CONCENTRATION IN THE URINE WHEN INJECTED BY RECTUM.

By T. M. CARPENTER and E. B. BABCOCK.

(From the Carnegie Nutrition Laboratory, Boston.)

Studies were made with four medical students of the absorption of ethyl alcohol when introduced by the rectum. Concentrations of 5, 7.5, and 10 per cent were used and amounts varying from 200 to 1,000 cc. were introduced into the rectum. The average amount of alcohol given was about 25 gm. The solutions were retained from 2 to 5 hours. The unabsorbed alcohol was determined in the washouts by the Nicloux method. The absorption of alcohol was practically complete in all cases, the highest amount unabsorbed found being about 0.5 gm.

The concentration of alcohol in the urine was also determined by collecting the urine in as short periods as possible after the alcohol was introduced into the body either by mouth or by rectum. The maximum concentration was about 0.40 mg. per 1 cc. and appeared between the 1st and 2nd hours after ingestion. It fell to zero in about 5 to 6 hours. In one subject there was a tendency for the alcohol percentage to be higher when it was taken by mouth; in the other the concentrations were about the same irrespective of the method of ingestion.

THE COMPOSITION OF RENAL AND VESICAL CALCULI.

By SAMUEL BOOKMAN.

In the quantitative analysis of fourteen stones, it is proven that the alkalinity of the urine is probably at no time sufficiently strong to dissolve the uric acid and urate stones, neither is it acid enough to cause the solution of oxalate and phosphate stones, which will account for the formation of mixed stones. The percentage composition of these stones exemplifies the change from the acid to the alkaline precipitation, or the reverse, the phosphates and carbonates being precipitated in the alkaline condition, and the urates and uric acid in the more acid reaction, the oxalates in the less acid urines, as well as in the alkaline condition. No doubt the disturbance in the colloid equilibrium plays an important part in this precipitation, but in addition we have the following contributing conditions to reckon with as well: diet, acidity, concentration, inflammation, and bacterial decomposition within the urinary tract.

THE ACTIVE CONSTITUENT OF THE THYROID: ITS ISOLATION, CHEMICAL PROPERTIES, AND PHYSIOLOGICAL ACTION.

By E. C. KENDALL.

(*From the Mayo Clinic, Rochester, Minn.*)

Primary cleavage of thyroid proteins results in acid-insoluble A and acid-soluble B constituents. Separation of the iodine compound from A is accomplished by the solubility of its barium compound in barium and sodium hydroxides. Many successive precipitations, alternated with heating in sodium hydroxide and the action of carbon dioxide, remove impurities, the last traces of which are removed by dissolving in alkaline alcohol and precipitating with acetic acid. The active constituent separates in microscopic needles. It may be precipitated as free base or in salt form. The free base is insoluble in alcohol and contains 65 per cent of iodine. The sulfuric acid salt is soluble in alcohol and contains 60 per cent of iodine. Its molecular weight appears to be 586. The chemical properties depend upon the degree of purification and the presence of other substances. In impure form heating with acid destroys the physiological activity and gives a product resembling Baumann's iodothyrin.

The physiological activity depends upon the amount administered and the susceptibility of the individual. 0.125 to 0.25 mg. daily is sufficient for cretinism. The maximal quantity tolerated by human beings so far tried has been 2 mg. daily. The response is not immediate; 24 to 72 hours may elapse before the physiological effects appear. So called hyperthyroidism symptoms and death follow administration of excessive doses to animals.

NEW BUFFER MIXTURES, STANDARD TUBES, AND COLORIMETER FOR DETERMINING THE HYDROGEN ION CONCENTRATION OF SEA WATER.*

By J. F. McCLENDON.

(From the Physiological Laboratory of the University of Minnesota, Minneapolis.)

Two stock solutions were used: (1) 0.3 M boric acid containing 2.25 per cent NaCl, and (2) 0.075 M borax containing 1.9 per cent NaCl, and mixed in thirty different proportions that may be estimated by plotting from data in the following table. The first column gives the percentage of the boric acid solution, the second that of the borax solution, the third the P_H of sea water of 13.9 gm. Cl per liter, the fourth the P_H of sea water of 17.7 gm. Cl per liter, and the fifth the P_H of sea water of 21.5 gm. Cl per liter. The tubes of borate mixtures and sea water have been calibrated fifty times by means of the hydrogen electrode, and recalibrated by the same means at Princeton University.

79.5	20.5	7.50	7.45	7.40
72	28	7.70	7.65	7.60
68	32	7.80	7.75	7.70
60	40	8.00	7.95	7.90
37	63	8.50	8.45	8.40
8	92	9.00	8.95	8.90
1	99	9.10	9.05	9.00

Thymolsulfophthalein was used as indicator from P_H 7.9 to 9 and *o*-cresolsulfophthalein from P_H 7.5 to 8.3, so as to form 0.001 per cent solution in both buffers and sea water. The buffers were sealed in "Nonsol" tubes of 24 mm. bore and the sea water was placed in open tubes of the same bore, and compared in a colorimeter having the lenses of a stereoscope placed in contact so as to bring the centers of the tubes together in a sharp line when viewed from the side with one eye 6 inches from the lenses. Tables were prepared for determining the CO_2 tension, CO_2 content, O_2 content, and heat given off in the respiration of marine animals.[9]

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* Read by title.

THE INFLUENCE OF TEMPERATURES ABOVE FREEZING ON THE CHANGES IN CHEMICAL COMPOSITION, BACTERIAL CONTENT, AND HISTOLOGICAL STRUCTURE OF THE FLESH OF THE COMMON FOWL.

By M. E. PENNINGTON, J. S. HEPBURN, E. Q. St. JOHN, and E. WITMER.

(*From the Food Research Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.*)

Chickens were killed and picked, and were kept at a temperature of approximately 0°C. for 24 hours, in order to remove the animal heat. Some birds were then analyzed as "freshly killed chickens;" others were kept in the room (average temperature 23.9°C., maximum holding period 4 days), or in a house refrigerator (temperature 7.2–12.8°C., maximum holding period 7 days), or in a mechanically refrigerated "chill room" (temperature 0°C., maximum holding period 3 weeks), and were then analyzed. A number of analyses were made, both of freshly killed birds and of birds kept at each of the temperatures stated. While the total nitrogen content of the muscle remained unchanged, the partition of the nitrogen underwent changes at all three temperatures of holding. Thus the total nitrogen of the aqueous extract invariably increased in the chickens kept in the room, the increase being a direct function of the period of holding; in the refrigerator this fraction of the nitrogen at times either increased or decreased, depending on the period of holding; in the chill room it decreased during the first 2 weeks of holding, then increased. At all three temperatures of holding, the amino-acid and basic nitrogen increased at the expense of the protein nitrogen, usually in a progressive manner. The most striking change in the fat constants

[9] The sealed tubes and colorimeter may be obtained from Hynson, Westcott, and Dunning, Baltimore.

was an increase in the acid value, which became progressively greater as the period of holding lengthened. Both the formation of amino-acid and basic nitrogen, and the increase in the acid value of the fat occurred most rapidly at the temperature of the room, least rapidly at that of the chill room. Frequently the saponification number and the Hehner number of the fat increased or decreased *simultaneously*. The bacteria, which grow under aerobic conditions, increased in both the muscles and the skin at all three temperatures of holding; the increase was enormous in the chickens kept in the room, and was less marked, though distinct, in those kept in the refrigerator, and in the chill room; the increase usually became greater as the period of holding lengthened. Organisms which liquefy gelatin were comparatively few in number, even in the chickens which had been kept in the room, and in which the proteolysis had become most advanced. The muscle cells changed in width at all three temperatures of holding. A pronounced swelling or increase in width occurred at the temperature of the room, and a slight increase in width at the temperature of the refrigerator. A shrinkage or decrease in width of the muscle cells—usually progressive—took place at the temperature of the chill room. At all three temperatures, room, refrigerator, and chill room, rupture of the muscle fibers and exudation of the cell contents occurred; the muscle, its nerves and blood vessels, and the connective tissue became altered in histological structure and in the staining power of their various elements; these changes in structure and in affinity for stains depended in part on the temperature, and in part on the time of holding.

The changes which occurred during holding in the chill room for 3 weeks were about equal to those occurring during holding in the house refrigerator for 5 to 7 days. The changes which took place during holding in the house refrigerator for 5 days were less than those taking place during holding in the room for 2 days.

THE INFLUENCE OF TEMPERATURES BELOW FREEZING ON THE CHANGES IN CHEMICAL COMPOSITION, BACTERIAL CONTENT, AND HISTOLOGICAL STRUCTURE OF THE FLESH OF THE COMMON FOWL.

By M. E. PENNINGTON, J. S. HEPBURN, E. Q. St. JOHN, and E. WITMER.

(*From the Food Research Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.*)

Chickens were killed and dry picked and kept at 0°C. for 24 hours to remove the animal heat. They were next frozen solid by holding for 48 hours in a "sharp freezer" at a temperature not higher than -14.4°C., then were stored in mechanically refrigerated "freezers" at a temperature of -9 — -13°C. for definite periods of time, the maximum period being 2 years. Every 3 or 4 months, several samples were taken from the freezer and analyzed. The "freshly killed chickens" of the preceding research were taken as the fresh standards with which to compare these hard frozen chickens. The muscular tissue showed a progressive desiccation, or loss of water, while the chickens were held in the freezer. The total nitrogen of the muscle remained unchanged; however, changes occurred in the partition of the nitrogen. Thus the total nitrogen of the aqueous extract increased or decreased, depending on the period of holding. The amino-acid and basic nitrogen showed small but progressive increases; this indicated that a slow but distinct proteolysis had occurred, leading to the accumulation of non-protein nitrogenous compounds at the expense of the protein. The acid value of the fat increased; the saponification number and the Hehner number decreased during the early months of holding, then increased. The hydrolysis of the fat and the digestion of the protein occurred far less rapidly than in chickens held at temperatures above freezing.

The bacteria, which grow under aerobic conditions, decreased in number in the muscle during the freezer storage; the decrease was apparent at the end of 4 months, became more marked as the period of holding lengthened, and was most marked after the birds had been in the freezer for 1 year or longer. Organisms which liquefy gelatin and those which grow in an anaerobic environment were present only occasionally in the chickens from the freezer; organisms of putrefaction, which grow under anaerobic conditions after their suspension in physiologic salt solution has been kept at 80°C. for 10 minutes, were present still less frequently. The evidence points to an enzymic rather than to a bacteriologic cause for the foregoing chemical changes.

The phenomenon of freezing in itself produced little, if any, change in the width of the muscle cells, as was shown by measurement of sections cut from different pieces, removed from the same muscles before and after freezing the chickens at an average temperature of −11°C. in the "freezer." However, during prolonged holding in the freezer, a progressive shrinkage or decrease in width of the cells occurred. Muscle fibers with a ruptured sarcolemma and exuding cell contents became more numerous as the period of holding lengthened. Characteristic changes took place in the histological structure of the muscle cells, nerve fibers, blood vessels, and connective tissue, and in the affinity of their elements for stains. When chickens were stored in the freezer in closed metallic containers, desiccation was prevented; the other changes were retarded but not prevented. The changes here recorded were most marked *after* the normal commercial period of freezer storage had been exceeded. Both the analyses and organoleptic tests showed that no loss of food value had occurred while the chickens were in the freezer; the birds were still wholesome and nutritious; the only appreciable change was a loss in flavor after holding in the freezer for a period greater than 9 months.

The changes in chemical composition during holding in the freezer for 1 year were about equal to the changes which occurred during holding in the chill room for 3 weeks.

AUXO AMYLASES.

By ELBERT W. ROCKWOOD.

(*From the University of Iowa, Iowa City.*)

Many amino-acids increase the hydrolytic cleavage of starch by ptyalin. This is true of the α-amino-acids such as glycocoll and tyrosine which result from the hydrolysis of proteins. The accelerating effect is not lost by substituting other radicals for hydrogen of the amino group, as in hippuric acid. Where the amino group is substituted in the carboxyl the compound has no stimulating effect, as in acid amides including urea. Introduction of the sulfonic radical neutralizes the effect of the amino group, as in sulfanilic acid. The position of the amino group, as in the aminobenzoic acid isomers, does not modify their activity; all stimulate the ptyalin. Compounds of amino-acids, like the proteins, also increase the amylolytic activity of ptyalin. As far as has been determined the pancreatic amylase is similarly affected by the amino-acids. The work is being continued.

A CHEMICAL STUDY OF PROLONGED INANITION.

By R. E. SWAIN.

(*From Leland Stanford Junior University, California.*)

The subject studied was a tailor by occupation, 30 years of age, who voluntarily subjected himself to a period of starvation which was continued for 60 days. The body weight was about 61 kilos at the beginning of the fast and about 36 kilos at the end. The excreta were collected for analysis from the 40th to the 60th days, and at intervals during this period the body temperature, pulse rate, blood counts, and other clinical data were recorded. The total urinary nitrogen output was maintained at a higher level than that recorded in previous cases of fasting, and shows a rise from the 53rd to the 56th days similar to the premortal rise in lower animals. This rise was followed by a rapid decrease in the daily excretion for the rest of the period. Ammonia is relatively very low. Creatinine was fairly constant until the 54th day when, following the rapid increase in the total nitrogen output and subsequent rapid fall, it decreases in amount to the end of the fast. Creatine is relatively high, exceeding the creatinine in amount during the period of high total nitrogen.

ADENINE-URACIL DINUCLEOTIDE AND THE STRUCTURE OF YEAST NUCLEIC ACID.*

By WALTER JONES and B. E. READ.

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*Published in full in *J. Biol. Chem.*, 1917, xxix, 111.

STUDIES OF AUTOLYSIS.

V. THE INFLUENCE OF BILE ON AUTOLYSIS.*

By H. C. BRADLEY and JOSEPH TAYLOR.

THE COMPOSITION OF ADIPOCERE.**

By R. F. RUTTAN and M. J. MARSHALL.

THE USE OF COTTON SEED AS FOOD.†

By THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

CYSTINE AND THE MINIMUM PROTEIN REQUIREMENT OF THE DOG.

By HOWARD B. LEWIS.

DEMONSTRATIONS BEARING ON COLORIMETRIC CREATININE AND NITROGEN DETERMINATIONS.

By OTTO FOLIN, E. A. DOISY, RICHARD BELL, and W. DENIS.

A NEW TEST FOR THE DETECTION OF OCCULT BLOOD IN STOOLS.‡

By W. G. LYLE.

THE PRODUCTION OF LACTIC ACID BY INTRAVENOUS INJECTION OF ALKALI.

By J. J. R. MACLEOD.

OBSERVATIONS ON THE FATE OF INOSITE IN THE DOG.

By ISIDOR GREENWALD and MORRIS WEISS.

ALKALINITY AND CARBONIC ACID IN SEA WATER.

By L. J. HENDERSON and E. J. COHN.

SOME EXPERIMENTS ON PROTEOLYTIC ACTIONS.‡

By E. M. FRANKEL.

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*Published in full in *J. Biol. Chem.*, 1917, xxix, 281.

**Published in full in *J. Biol. Chem.*, 1917, xxix, 319.

†Published in full in *J. Biol. Chem.*, 1917, xxix, 289.

‡Read by invitation.

THE KINETICS OF INVERTASE ACTION.*

By J. M. NELSON and W. C. VOSBURGH.

DEMONSTRATION OF A PERFUSION PUMP.**

By F. B. KINGSBURY.

VIRTUAL SUGAR.

By HUGH McGUIGAN.

THE DETERMINATION OF HEAVY METALS IN PHYSIOLOGICAL TISSUES AND FLUIDS.

By SAMUEL BOOKMAN.

A METABOLISM STUDY OF MANITOU (ALKALINE) MINERAL WATER.

By HELEN I. MATTILL and H. A. MATTILL.

THE REARING OF CHICKS UNDER LABORATORY CONDITIONS WITH ISOLATED FOOD SUBSTANCES.

By M. E. JAFFA and H. A. MATTILL.

THE INORGANIC COMPOSITION OF THORACIC DUCT LYMPH.†

By A. B. MACALLUM.

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*Read by invitation.

**See Kingsbury, F. B., *J. Biol. Chem.*, 1916-17, xxviii, 167.

†Read by title.

ABSTRACT OF THE EXECUTIVE PROCEEDINGS OF THE ELEVENTH ANNUAL MEETING.

NEW MEMBERS: T. B. Aldrich, Joseph C. Bock, Glenn E. Cullen, E. M. Frankel, Frank C. Gephart, A. O. Gettler, Albert G. Hogan, C. S. Hudson, E. Newton Harvey, Franklin C. McLean, Sergius Morgulis, Walter W. Palmer, C. J. V. Pettibone, Anton R. Rose.

AMENDMENTS TO THE CONSTITUTION: Article IV, Section 1-B, was amended to read:

> "The annual meeting shall be held at a time and place chosen by the Council in consultation with the Executive Committee of the Federation of American Societies for Experimental Biology."

Article V, Sections 4-D, 4-E, 4-F, and 5-B were amended to read:

> Section 4-D: "At the opening of the first executive session of the ensuing annual meeting the Secretary shall formally present the regular nominations for the Nominating Committee."
>
> Section 4-E: "Additional nominations for the offices and for membership in the Council may be made by any member at the opening of the first executive session of any annual meeting."
>
> Section 4-F: "Nominations for membership on the Nominating Committee shall be made by or for individual members, either in person or by proxy, and not otherwise, at the opening of the first executive session of each annual meeting."
>
> Section 5-B: "All elective officials shall be selected by ballot at the close of the first executive session of each annual meeting."

Article IX, Section 3-C was amended to read:

> "Affirmative votes from three-fifths of the members voting shall be required for the adoption of an amendment."

OFFICERS FOR THE YEAR 1917: *President,* Carl L. Alsberg; *Vice-President,* Albert P. Mathews; *Secretary,* Stanley R. Benedict; *Treasurer,* Harold C. Bradley. *Additional Members of Council,* Lawrence J. Henderson, Philip A. Shaffer, Frank P.

Underhill. *Nominating Committee*, W. R. Bloor, Wm. J. Gies, Philip B. Hawk, Graham Lusk, E. V. McCollum, Lafayette B. Mendel, Victor C. Myers, D. D. Van Slyke, Carl Voegtlin.

DUES: The annual dues for the year 1917 were fixed at $2.00.

STANLEY R. BENEDICT,
Secretary.

PROCEEDINGS

OF THE

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

TWELFTH ANNUAL MEETING
Minneapolis and Rochester,
Minnesota

December 27-29, 1917

ABSTRACTS OF PAPERS
ABSTRACT OF EXECUTIVE PROCEEDINGS

Volume IV
No. 2

PROCEEDINGS OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS.

TWELFTH ANNUAL MEETING.

Minneapolis and Rochester, Minn., December 27–29, 1917.

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For the List of Members and the Constitution of the Society see the Register of the Federation of American Societies for Experimental Biology comprising the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, and the American Society for Experimental Pathology.

THE RÔLE OF SOME INORGANIC ELEMENTS IN NUTRITION.

By LAFAYETTE B. MENDEL and THOMAS B. OSBORNE.

(*From the Laboratory of the Connecticut Agricultural Experiment Station and the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven.*)

The relative importance of the individual inorganic salts was long ago determined for plants, but hitherto no such definite knowledge of the requirements of animals has been possible owing to the failure of animals to thrive on a diet of purified foodstuffs. Now that the essential factors of a successful dietary have been ascertained it has become possible to prepare rations in which the individual ions are excluded except for the unavoidable small amounts present in the products used to supply the water-soluble vitamine or found as impurities in the other ingredients of the food.

From experiments with such diets it has been demonstrated that calcium and phosphorus are needed in considerable amounts; for with only a trace of either of these elements in the food, rats failed to grow and sooner or later declined. On diets in which only supposedly insignificant quantities of magnesium, sodium, potassium, or chlorine were present growth at a normal or nearly normal rate was made for about 1 year. Where both sodium and potassium were present in the above small amounts no growth occurred.

THE NUTRITIVE PROPERTIES OF KAFIR.

By ALBERT G. HOGAN.

(*From the Department of Chemistry, Kansas Agricultural Experiment Station, Manhattan.*)

Our investigations of the nutritive properties of kafir were interrupted, and as some time may elapse before this study can be resumed we deem it advisable to take this opportunity of making a preliminary report. Although we have a high degree of confidence in the correctness of our conclusions, these observations are not to be considered as necessarily final.

When kafir forms the sole diet of white rats, it has about the same nutritional value as corn. Kafir is seriously deficient in calcium, but when this element (Ca lactate) is supplied, the animals grow and attain maturity, though the growth period is considerably prolonged. Growth on kafir and ash mixture is apparently more rapid than on corn and ash mixture.

Females receiving kafir and a salt mixture became pregnant and delivered young, but in no case did these litters survive more than a few days.

The proteins of kafir seemed to have a biological value equal to that of corn in inducing growth.

Kafir is probably deficient in one of the accessory substances. A comparison of the data obtained by feeding kafir and by feeding corn indicates that the fat-soluble A, as described by McCollum, is a limiting factor in kafir.

Our investigations on the nutritive properties of kafirin were brought more nearly to completion and the data has been published in detail.[1] Kafirin, when it forms the sole source of protein in the diet, is apparently inadequate for growth or for the maintenance of young animals (rats). Lysine is the first limiting factor of kafirin, and cystine is the second. The results indicate that lysine is indispensable not only for growth, but also for the maintenance of young animals.

THE RELATION BETWEEN PHOSPHORUS METABOLISM AND THE SECRETION OF MILK FAT.

By EDWARD B. MEIGS and N. R. BLATHERWICK.

(From the Bureau of Animal Industry, United States Department of Agriculture, Washington.)

Blood samples have been obtained nearly simultaneously from the jugular and mammary veins of milking cows, and the plasma from such samples has been analyzed for total, lipoid, and inorganic phosphorus, the object being to determine whether the concentrations of these kinds of phosphorus were appreciably changed during the passage of the blood through the active gland.

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[1] Hogan, A. G., *J. Biol. Chem.*, 1918, xxxiii, 151.

In the earlier experiments the cow was disturbed by precautions which were afterward found to be unnecessary, and the results of the experiments differed according as the subjects were much or little disturbed before and during the collection of the mammary blood. When they were much disturbed, the mammary plasma had the same concentration of lipoid phosphorus as the jugular, but a higher concentration of inorganic phosphorus, and a correspondingly higher concentration of total phosphorus. When they were little disturbed, the mammary plasma had a lower concentration of lipoid phosphorus and a higher concentration of inorganic phosphorus than the jugular.

These results are taken to mean that the precursor in plasma of both milk fat and milk phosphorus is either lecithin or some related compound. The ratio phosphorus: fat in lecithin is about 1: 18, whereas it is a out 1: 50 in milk. If, therefore, the mammary gland takes from the plasma enough lecithin or enough of some related phosphatide body to supply its milk with fat, it gets with it more phosphorus than can be used for the milk, and the excess must be returned to the blood. These considerations are taken to explain the back-flow of inorganic phosphorus from the mammary gland to the blood, which occurred in all the experiments. The experiments indicate further that the disturbance of milking animals interferes with the taking up of phosphatide by the mammary gland, but not with the back-flow of inorganic phosphate from the gland to the blood.

THE EFFECT OF COTTONSEED FLOUR ON ANIMALS, WITH PARTICULAR REFERENCE TO THE INORGANIC CONSTITUENTS OF WHITE RATS.

By F. C. COOK.

(From the Bureau of Chemistry, United States Department of Agriculture, Washington.)

Six separate groups of animals, each group consisting of twenty-four rats, twelve mice, and six guinea pigs, were fed for periods up to 6 months on (1) 25 per cent cottonseed flour bread, (2) 25 per cent cottonseed flour dough, (3) 40 per cent cottonseed flour bread, (4) white flour bread, (5) entire wheat bread, and (6) mixed diet.

The exclusive bread diets did not support life, although no pathological lesions were found to be characteristic of any of the diets. 25 per cent cottonseed flour dough supported life longer than 25 per cent cottonseed flour bread. The animals fed entire wheat bread and white bread lived no longer than the animals fed the cottonseed flour breads.

Less nitrogen was required to maintain rats and mice at constant weight when white bread was fed than when entire wheat bread was fed. The largest amount of nitrogen required to maintain constant weight was in the case of the animals fed 40 per cent cottonseed flour bread. These findings are in line with the results reported in the literature for larger animals.

After 6 months, when the experiment closed, the bodies of twenty-four rats, four from each of the six groups, were separately analyzed for inorganic constituents.

The 40 per cent cottonseed flour bread was highest in total ash, P_2O_5, CaO, and MgO of any of the breads fed. The ash constituents were apparently not properly balanced as the bodies of the rats fed on this bread were low in total ash and especially in P_2O_5-free ash. The ratio of MgO to CaO was 1: 15 for the bodies of the rats fed on 40 per cent cottonseed flour bread and 1: 25 for the controls.

The exclusive feeding of cottonseed flour bread apparently caused changes in the ash constituents of the tissues of the rats analyzed. These changes appeared in the results for the rats fed 25 per cent cottonseed flour bread and dough and were marked in the cases of the rats fed 40 per cent cottonseed flour bread.

BORON. ITS EFFECT ON CROPS AND ITS DISTRIBUTION IN PLANTS AND SOILS IN VARIOUS PARTS OF THE UNITED STATES.

By F. C. COOK and J. B. WILSON.

(*From the Bureau of Chemistry, United States Department of Agriculture, Washington.*)

Borax and colemanite (calcium borate) were found to be effective larvicides for the house-fly.[2] To determine the effect of the boron, added to manure for this purpose, on plant growth a study was made extending over 3 years in which crops were grown in different parts of the country on soil fertilized with manure containing definite amounts of added borax and of added colemanite. In order to test the cumulative effect of the boron the same plants were treated with manure and boron for three seasons. Wheat, rye, oats, beets, tomatoes, cowpeas, lettuce, soy beans, string beans, potatoes, spinach, kale, cabbage, turnips, corn, and peach trees were included in the experiments.

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[2] Cook, F. C., Hutchison, R. H., and Scales, F. M., *U. S. Dept. Agric., Bull.* 118, 1914.

Practically the same amount of boron was absorbed by the plants whether it was added to the soil as soluble borax or as insoluble colemanite. Wheat, oats, and rye absorbed little boron, while leguminous and succulent plants absorbed comparatively large amounts. There was a marked difference in the distribution of the boron in the roots, tops, and fruit of the different plants. Leguminous plants were very sensitive to the boron. At Bethesda, Maryland, 0.0044 per cent H_3BO_3 added as borax and 0.0058 per cent added as colemanite to the upper 6 inches of soil caused no injury to lettuce, spinach, and kale, while at Arlington, Virginia (5 miles distant), the same amounts of boron gave a reduced yield of these crops. A similar difference in the effect of a definite amount of boron on other plants when grown on different soils is indicated. There is a decided difference in soils in rendering the added boron non-toxic, although all soils tested gradually rendered the soluble boron insoluble and non-toxic. In many soils there is a tendency for plants to absorb boron in proportion to the amounts added to the soil while in other soils the same amounts of boron were absorbed irrespective of the amounts added. The absorption and the toxic effect of boron on plants varies with the variety of plants, the solubility of the boron compound, the amount of the boron compound added to the soil, the time elapsing after the compound is mixed with the soil before planting, the amount of rainfall, etc., and finally with the character of the soil to which the boron compound is added.

THE INFLUENCE OF DIET ON THE URINARY CALCIUM AND MAGNESIUM EXCRETION OF MAN.

BY MAURICE H. GIVENS.

(*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.*)

Nelson and Burns[3] found that eight out of twenty-five persons, on random diets, excreted more magnesium than calcium in the urine. If one accepts the generally quoted statement that calcium and magnesium excretion is dependent upon the quality and quantity of nourishment ingested, their findings ought to be corroborated with subjects on diets having a content of magnesium greater than that of calcium. Accordingly nine healthy individuals consumed foods estimated to contain more magnesium than calcium for periods of 6 to 9 days. In only three cases was the urinary magnesium excretion greater than that of calcium and in no one of them was the excess of magnesium over calcium greater than 30 mg. per day. In two of these three individuals the urinary calcium output became greater than the magnesium by the addition to the daily intake of two glasses of milk; in the third case a similar result was obtained with a small dose of calcium lactate. When milk was added to the régime the calcium excretion was increased at once with a slight rise in the magnesium.

ACIDOSIS IN RELATION TO PANCREATIC DIABETES.

BY V. W. JENSEN.

(*From the Northwestern University Medical School, Chicago.*)

Elias[4] states that the introduction of acids into rabbits and dogs leads to hyperglycemia and glycosuria. Watanabe[5] in a recent article had occasion to test this out on rabbits and concludes that the results are not at all uniform, and he found that the amount of sugar in the blood was not perceptibly increased. He did not test the urine, but there was no reason to believe that there would have been any glycosuria, because of the low amount of sugar in the blood.

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[3] Nelson, C. F., and Burns, W. E., *J. Biol. Chem.*, 1916–17, xxviii, 237.

[4] Elias, H., *Biochem. Z.*, 1913, xlviii, 120.

[5] Watanabe, C. K., *Am. J. Physiol.*, 1917, xlv, 30.

The present investigation was commenced without knowledge of this latter work and with the idea of deciding whether or not acid is a cause of glycosuria or a result of a diabetic condition. A great criticism of Elias' work is that the acid he used was too strong, 0.25 N HCl. It is quite possible that his results will hold good, but they are open to the objection that it might have been an indirect action on the pancreas, or a corrosive action on the duodenum.

In the present work, I used 0.1 N HCl, 600 cc. given in 200 cc. doses three times a day. This acid is close to the strength normally found in the dog's stomach. This was tried on two dogs. One dog was rendered glycosuric by removing seven-eighths of the pancreas, and the other highly sensitized by removing six-sevenths of the pancreas. The sensitized dog in no case gave glycosuria by addition of acid, although he could have been rendered highly glycosuric on carbohydrate diet, but was free from it on a meat diet. The glycosuric dog ran on the average 7 gm. of sugar a day on meat diet. During the acid period this amount was doubled. When an equal amount of sodium carbonate was given, the sugar amount dropped to 0.25 gm. per day.

While these experiments are too few in number to give any definite conclusions, it would seem at the present time that acidosis is a result of a glycosuric condition and that after it has developed it acts as a vicious circle and increases the condition. This statement is supported by the fact that when a non-glycosuric sensitized dog is fed a carbohydrate diet, he eliminates 25 gm. of sugar per day, and the acid in urine is doubled, and ammonia excretion is greatly increased.

THE ACTION OF AMMONIUM COMPOUNDS ON PTYALIN.

By ELBERT W. ROCKWOOD.

(From the Department of Chemistry, University of Iowa, Iowa City.)

Digestion was carried on in a medium neutral to litmus. Both salts of inorganic and organic acids were tested. The ammonium salts of the inorganic acids increased the activity of the ptyalin as did also those of the stronger organic acids. In general the ammonium salts of the strong acids had a greater activating effect than those of the weak acids. To this oxalic acid is an exception, having little or no effect.

LIPEMIA.

By W. R. BLOOR.

(From the Biochemical Laboratory of Harvard Medical School, Boston.)

A study was made of the blood lipoids in a case of severe diabetic lipemia where the blood lipoids reached a very high value (over 13 per cent). Analyses were made of blood samples of which the first was taken 36 hours after the last meal and the others at intervals for about 30 days. In the first 20 days of this period no fat was eaten and the total food intake did not exceed 350 calories per day. In the beginning there was marked "diabetic anemia," the blood corpuscle percentage in the first sample being 29 per cent while the normal for this individual was about 42 per cent. During the first 5 days there was considerable acidosis—low CO_2 tension in the alveolar air, high urinary ammonia, and considerable quantities of acetone bodies in the urine—and during this period there was very little absorption of lipoids from the blood; in fact, as a result of the disappearance of the "anemia" and the accompanying concentration of the plasma the lipoid values became higher than at the beginning. The acidosis disappeared during the next 5 day period and with its disappearance there was a rapid lowering of the blood lipoids—about 50 per cent of the total lipoids in the 5 days. Of the lipoids, the fat decreased most rapidly, the lecithin next, and the cholesterol least. The rate of disappearance of the cholesterol was fairly uniform throughout, not being much affected by the presence or absence of acidosis. Changes in the fat and lecithin content of the blood corpuscles were marked throughout, while the cholesterol content remained quite constant, bearing out the earlier findings that the blood corpuscles take an active part in the metabolism of fat and lecithin but not of cholesterol. During the remainder of the period of examination the blood lipoids continued to decrease slowly. The addition of 50 to 60 gm. of fat to the daily diet and the increase of the diet to over 1,000 calories produced only a slight lessening of the rate of decrease.

FURTHER STUDIES IN AUTOLYSIS.

By H. C. BRADLEY.

(*From the Laboratory of Physiological Chemistry, University of Wisconsin, Madison.*)

I. Brain.—The autolysis of nervous tissue is accelerated by acids and inhibited by alkali just as are other gland tissues. While the amount of protein present in nervous tissue is very small, the rapidity of its autolysis is about equal to that of liver or kidney. Because of the exceedingly delicate protein framework of the nerve cell, its destruction through the autolytic mechanism may quickly proceed to an irreparable extent, while other organs with their large protein content may undergo large atrophic changes without irreparable damage.

II. Muscle.—Of the three types of muscle tissue, striated autolyzes most slowly and to the smallest extent. Cardiac and smooth muscle autolyze from 50 to 100 per cent faster. All three are found to be accelerated by acidity and inhibited by alkalinity, but to a much less striking extent than the gland tissues previously studied.

III. Colloids.—The work of Ascoli and Izar on the accelerating action of inorganic hydrosols on autolyzing liver was repeated, but not confirmed. Sols of silver, gold, and platinum, prepared by the Bredig method, were found to be wholly inert, neither accelerating nor inhibiting autolysis. Colloidal $Fe(OH)_3$, As_2S_3, and Sb_2S_3 when dialyzed free from electrolytes were found equally inert when added to autolyzing liver pulp. The disagreement between these results and those of the above mentioned authors is so complete that further experiments are under way.

THE INFLUENCE OF MECHANICAL WORK UPON PROTEIN METABOLISM DURING THE HEIGHT OF MEAT DIGESTION IN THE DOG.

By H. V. ATKINSON.

(*From the Physiological Laboratory, Cornell University Medical College, New York City.*)

A dog was given 600 gm. of meat daily in one portion, and the nitrogen eliminated in the urine during the 4th, 5th, and

6th hours was determined. The dog was then caused to run on a treadmill during the 5th hour and it was found that the quantity of nitrogen in the urine was the same as during rest. Therefore, mechanical work has no influence on the rate of absorption per hour or the intensity of protein metabolism per hour in a dog which has been given meat in large quantity.

METABOLIC STUDY OF α-UREIDOISOBUTYLACETIC ACID.

By ALICE ROHDE.

(From the Hooper Foundation, San Francisco.)

The procedure described for the identification of α-ureido-β-phenylpropionic acid in the presence of amino-acids and of urea is found to be applicable to the identification of α-ureidoisobutylacetic acid under similar conditions. This uramino-acid was recovered in considerable quantity from the urine of a cat after slow, continuous injection of a saline solution of the material into the blood stream of the animal.

CHANGES IN THE COMPOSITION OF MUSCLE OF SALMON DURING MIGRATION.

By CARL H. GREENE.

(From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.)

The muscles of the salmon during the fast of the spawning migration show a great decrease in the amount of fat and protein present, with a corresponding increase in the water content. During the first part of the migration the fat shows the greatest decrease but with the beginning of the disappearance of the fat the protein catabolism becomes marked. On the spawning grounds the fish show great exhaustion of all food reserves in comparison to the initial condition.

The organic extractives as a whole and the extractive nitrogen in particular show a general tendency to increase during the migration fast. There is no apparent relationship in the behavior of the organic and inorganic extractives.

The amino nitrogen in the fat-free tissue increases from 61 to 84 mg. per 100 gm. This increase is most marked calculated on the basis of the relation between the amino nitrogen and the protein from which it must be formed. Proportionately, the increase is nearly 100 per cent. During the migration the amino-acids are liberated at the expense of an ever increasing wastage of the muscle proteins.

The most significant change lies in the relation of the amino-acids to the water in the muscle. At the beginning the amino nitrogen value calculated per 100 cc. of water increases from 80 to 100 mg. Its concentration then remains unaffected by subsequent changes. With the stimulated protein catabolism the muscles, or more particularly the fluid in the muscles, become saturated with amino nitrogen early in the migration period. This saturation continues through the migration regardless of the great loss of muscle protein, until in the end the exhaustion of the organism proceeds to a degree culminating in death.

The determining factor in the behavior of the amino-acids would seem to be the volume of water present in the muscles and not the protein. This previously undescribed water volume effect is a strong regulating factor and must be added to those suggested by Van Slyke as probably controlling the amino-acid content of the muscles.

THE COMPOSITION OF THE OVARIES OF THE SALMON DURING MIGRATION.

By C. W. GREENE.

(From the Department of Physiology, University of Missouri, Columbia.)

Chemical analyses were made of the ovaries of fifteen individual salmon of the species *Oncorhynchus tschawytscha* chosen from five stations of the Columbia River basin during the migration to the spawning grounds. The analytical results, with the exception of the fat, were computed in terms of the fat-free sample, and yield the following comparisons. The neutral fat in the wet sample of ovarian tissue averages 14.15 per cent at the mouth of the river, 17.38 per cent about 210 miles up the river, and drops to 10.38 per cent at the spawning grounds. This fat is a superload in the ovules and cannot be considered a part of the ovarian protoplasm. Leaving the fat out of consideration and calculating on a fat-free or protoplasmic basis, the total proteins average 29.8 to 31.2 on the lower river, 31.3 in the upper river, and 29.4 at the spawning grounds, a remarkably constant composition. The lipoids decrease from an average of 4.8 at the mouth of the river to 3.3 at the upper river, and 2.85 on the spawning grounds, showing that the percentage of lipoids diminishes with development, at least in this fasting fish. The organic extractives slightly increase during the early stages of migration but decrease during late migration and at spawning. The inorganic salts increase with the migration. Summarizing, it is apparent that the reserve fat of the ovaries decreased with the migration as does also the percentage amount of lipoids present. The organic extractives vary in harmony with similar changes in other tissues of the body though less in amount. The protein content of this growing reproductive tissue remains remarkably constant. In view of the great increase in mass of the ovaries during the migration and fast, from 600 to 1,000 per cent increase, we must look to other tissues for the food supply used in the growth of the ovaries. Analyses already presented show that the muscles of the salmon contribute this material. The muscles decrease in absolute weight and also in percentage of protein and in fat during the migration. The most vital constituent is the protein. The muscle proteins are diminished by 30 per cent during the migration without affecting the physiological or histological characteristics of the tissue. So it is obvious that this great excess of protein must exist in the muscle in the form of stored protein. There is a protein storage in the salmon comparable to the similar storage of fats. This stored protein is available during the migration for the synthesis of new protein tissue in the developing ovaries.

THE PREPARATION OF YEAST NUCLEIC ACID.

By EMIL J. BAUMANN.

(From the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, and the Biochemical Department, University of Toronto.)

A new method for the preparation of yeast nucleic acid is suggested based on the facts: (1) that nucleoprotein is a type of acid protein and is separated into nucleic acid and protein by treatment with alkali in the cold; (2) that nucleic acid is soluble in dilute acetic acid; (3) that nucleic acid is precipitated by magnesium sulfate in the presence of hydrochloric acid, as proposed by Slade.

Fresh brewer's yeast is diluted with water and treated with a concentrated solution of sodium hydroxide (100 gm. of alkali per kilo of yeast) and the solution then partially neutralized with hydrochloric acid. The solution is made acid with acetic acid and filtered from the separated protein after the bulk of the precipitate has settled. The filtration is best accomplished with fluted filters or paper pulp. The clear filtrate is treated with 5 per cent of magnesium sulfate and enough hydrochloric acid to cause the nucleic acid to flock out. The precipitated material is the monomagnesium salt and may be dried after washing with alcohol and ether. Yield: 4–7.5 gm. per kilo of yeast.

The analyses for N, Mg, and P are lower than the calculated figures, but the ratios of the three elements correspond accurately with those for monomagnesium nucleate.

The method is applicable to animal nucleic acid with slight modifications.

STUDIES IN ENDOGENOUS URIC ACID METABOLISM.

By HOWARD B. LEWIS, MAX S. DUNN, and E. A. DOISY.

(*From the Laboratory of Physiological Chemistry of the University of Illinois, Urbana.*)

The influence of proteins and various protein derivatives upon the hourly uric acid elimination has been studied in the fasting subject on a purine-free low protein diet. Protein (egg white, cottage cheese) caused a slight rise beginning the 2nd hour after ingestion and increasing to a maximum at the 4th hour. Ingestion of equivalent amounts of nitrogen in the form of amino-acids (glycocoll, alanine) resulted in a sharp rise the 2nd hour after ingestion followed by a prompt return to the normal level. Administration of a second portion of glycocoll following the return to normal caused a like increase in the uric acid excretion. The increased uric acid metabolism was not apparently associated with the phenomena of specific dynamic action of the glycocoll, since aspartic and glutamic acids which probably exert no such action caused increases in the uric acid excretion of even greater magnitude than glycocoll or alanine. Neither urea nor ammonium chloride administered in amounts comparable to the amino-acids had any influence on the uric acid output. These results are believed to indicate that the rise in uric acid excretion following ingestion of protein food is not due mainly to the work of the digestive glands but to stimulation of the cellular metabolism by the amino-acids liberated in the digestion of the protein. Creatinine excretion was not affected in any of the experiments. Uric acid was determined colorimetrically according to Benedict and Hitchcock.

ASPECTS OF HIPPURIC ACID CONJUGATION.

By E. LACKNER, A. LEVINSON, and WITHROW MORSE.

(From the Nelson Morris Institute, Chicago.)

The liver is concerned with several types of pairing, yet Salkowski reported that the principal seat of hippuric acid conjugation is resident in the kidney. Salkowski's observations have been repeated by others, and variations in the sweeping statements given by him have had to be made. In the dog, Kingsbury found that there are other organs concerned with this type of pairing. The purpose of the following experiments was first of all to determine whether the liver played a recognizable rôle in hippuric acid synthesis, and, secondly, whether, if this is true, the principle could be utilized in detecting obscure liver involvements in man.

Two male dogs were isolated in metabolism cages and the food, water, and excretions observed over a period of 34 days. The urine was collected in 24 hour quantities. Hepatic involvement was made by the use of hydrazine sulfate, which, according to Underhill and Kleiner and to Gideon Wells, produced liver lesions while leaving the kidneys untouched. The truth of this is vouched for by the chloride figures of the present set of experiments as well as by the evidence of autopsy. Hippuric acid was determined by the Folin-Flanders method; chlorides were determined by the method of Volhard; gasometric amino nitrogen determinations were made upon the hydrolyzed urine to determine the glycocoll participation.

The following table gives the résumé of the experiments:

(1) Average normal excretion of hippuric acid nitrogen per 24 hours.

	gm.
Dog I	0.029
“ II	0.032

(2) Average excretion after the administration of 2 gm. of sodium benzoate by mouth.

Dog I	0.082
“ II	0.069

(3) Average excretion after administration of hydrazine sulfate by mouth.

Dog I	0.043
“ II	0.045

(4) Average after injection subcutaneously of 500 mg. of hydrazine sulfate.

Dog I	0.026
“ II	0.030

(5) Average after following this injection with 2 gm. of sodium benzoate by mouth.

Dog I	0.015
“ II	0.014

Averages of the two dogs.

Experiment 1	0.030
“ 2	0.075
“ 3	0.044
“ 4	0.028
“ 5	0.014

From these figures, it is evident that normally, an increase in hippuric acid nitrogen after giving sodium benzoate by mouth occurs; that after giving hydrazine by mouth, the hippuric excretion was not affected; that no effect was observed upon the administration of hydrazine subcutaneously, but that *after hydrazine injection followed by sodium benzoate by mouth, less hippuric acid is excreted.*

The kidneys gave every evidence of being normal, as judged by the chloride excretion day by day and also by the condition of the kidney at autopsy. Professor Oscar T. Schultz, director of the Institute, kindly performed the autopsy and reported that Dog II gave wholly normal histological pictures, while Dog 1 showed some glomeruli and interstitial capillaries engorged, but no other indication of lesion. The livers, on the other hand, showed marked fatty degeneration with central congestion and central necrosis. It is to be concluded, then, that in the dog *the excretion of hippuric acid varies with the condition of the liver, the kidney remaining constant.*

If it is true that, in the mammal, the liver participates to a major degree in hippuric acid pairing, we should be able to recognize the involvement of the liver by this means. Several normal children were studied, sodium benzoate being given by mouth, and the hippuric acid figures compared with those obtained upon days when no benzoate is given. Invariably there is an increase in hippuric acid excretion. In one case where there was extensive liver involvement (child of 11 years, mitral regurgitation, heart decompensated, liver greatly enlarged), no increase in hippuric acid excretion following administration of sodium benzoate was observed.

CONCLUSIONS.

1. In dogs, there is a marked participation of the liver in the pairing of hippuric acid.
2. This may be determined by administering subcutaneous injections of hydrazine and following these injections by administration of sodium benzoate by mouth, the hydrazine inducing fatty degeneration of the liver, while the kidney remains intact. There is a lessened output of hippuric acid under these conditions.
3. What appears, from a single crucial instance, to be a similar condition in man was observed.

A MODIFICATION OF THE LEWIS-BENEDICT METHOD FOR THE DETERMINATION OF SUGAR IN BLOOD.

By STANLEY R. BENEDICT.

(From the Department of Chemistry, Cornell Medical College, New York City.)

A study of the reaction between picric acid and glucose in alkaline solution was undertaken to find conditions under which this reaction will proceed to completion in dilute solution, thus avoiding the necessity of concentrating by boiling, as in the Lewis-Benedict procedure. The results have shown that in no possible concentration of sodium hydroxide is the depth of color directly proportional to the concentration of sugar. This alkali therefore cannot be employed. With increase of concentration of carbonate during the reaction there is increase in depth of color developed almost indefinitely, but high concentrations of carbonate lead to the occurrence of secondary reactions, so that the color developed is again not directly proportional to the concentration of sugar. Thus the concentration of carbonate cannot be increased to cause the reaction to go to completion in dilute solution. It was found, however, that in high concentration of picric acid (greater than a saturated aqueous solution) the reaction with glucose will be definite and quantitative in dilute solution in the presence of a moderate concentration of sodium carbonate. Details of the method thus developed will be published shortly.

A PRELIMINARY REPORT ON THE URINARY EXCRETION OF SUGAR IN THE NORMAL DOG.

By STANLEY R. BENEDICT and EMIL OSTERBERG.

(*From Department of Chemistry, Cornell Medical College, New York City.*)

The excretion of sugar in the urine of a normal dog upon various diets was studied by means of a new method for determination of sugar in normal urine. The technique will be published in detail shortly. The results of the study showed that sugar excretion in the normal dog bears no relation to urinary volume, but is dependent upon food ingestion and the nature of the diet. After food is eaten the curve of sugar excretion promptly rises, reaching a maximum usually in the 4th to 5th hour after the feeding. The curve then falls quite rapidly, reaching a low level during the early morning hours. The sugar excretion is less on a meat diet than on a diet rich in starch, but the curve of sugar excretion follows the same general curve in both cases.

A NEW FORM OF COLORIMETER.

By JOSEPH C. BOCK and STANLEY R. BENEDICT.

(*From Department of Chemistry, Cornell Medical College, New York City.*)

An accurate colorimeter has been developed in which the place of costly and difficultly obtainable prisms is taken by mirrors. The instrument will be described in detail later.

ADSORPTION PHENOMENA IN COAGULATION.

By G. H. A. CLOWES.

(From the Gratwick Laboratory, Buffalo.)

Physical quality and speed of formation of blood clot appear to depend upon the degree of dispersion of reacting colloids and their initial and final solution concentrations. Much previous work on coagulation is invalidated owing to failure to recognize the extent to which individual coagulation factors are influenced by very slight variations in hydrogen ion content of the medium in the absence of the buffer system.

Precipitated or colloidal barium sulfate, calcium oxalate, etc., may be used to fractionate coagulation factors. Prothrombin is strongly adsorbed by these substances. Adsorption is proportional to the degree of dispersion of the adsorbent and is dependent apparently upon presence of unsaturated lipoids like cephalin, thus indicating probable surface rôle of lipoids in coagulation processes and affording a quantitative method of estimating prothrombin and lipoid.

SALT METABOLISM IN DIABETES.

By A. H. BEARD and L. G. ROWNTREE.

(From the Department of Medicine, University of Minnesota, Minneapolis.)

An investigation was undertaken to determine the cause of the gain of weight in diabetes during starvation and on low carbohydrate intake. Gains up to 10 and 12 pounds were encountered during periods of low caloric intake. Edema developed in three cases in a series of twenty-five.

It was found that diabetic patients allowed to use table salt *ad libitum* consumed frequently very large quantities, as high as 40 gm. a day in one instance. Salt and fluid balance charts were constructed and correlated with a weight chart. In some cases it was shown that the gain in weight and development of edema were synchronous with, and in all likelihood dependent upon retention of salt and water.

In one case with restricted sodium chloride intake, but taking large quantities of sodium bicarbonate, retention of fluid and marked gain in weight and edema developed. On withdrawing sodium bicarbonate and allowing sodium chloride loss of weight occurred.

OBSERVATIONS ON THE PERMANGANATE TITRATION OF URIC ACID CONTENT OF BLOOD.

By J. LUCIEN MORRIS.

(*From the College of Medicine, University of Illinois, Chicago.*)

The determination of the small amounts of uric acid in blood by permanganate titration in acid solution is subject to a relatively large error. This is due to the continued reduction of permanganate after all uric acid has been oxidized. The transitory nature of the pink color end-point gives an error of only 2 to 3 per cent of the 2 mg. of uric acid in the titration following precipitation from urine as zinc urate. The error is 20 to 30 per cent of the 0.2 mg. of uric acid present in the usual quantity of blood taken for analysis. Oxidation of uric acid by permanganate in the slightly alkaline solution of sodium bicarbonate is a much less vigorous reaction and affords an opportunity of measuring the conclusion of the oxidation. The end-point used is the very delicate iodostarch color, which can be applied to the detection of an excess of permanganate because oxidation of potassium iodide does not take place in the weakly alkaline solution until all uric acid has been oxidized.

The precipitated zinc urate and zinc carbonate are dissolved in hydrochloric acid and disodium phosphate is added until all the zinc has been precipitated. To the resulting solution of uric acid in sodium phosphates, with which the zinc phosphate is present as a precipitate, 25 cc. of a saturated solution of sodium bicarbonate, 5 cc. of a 10 per cent potassium iodide solution, and 1 cc. of a 0.5 per cent soluble starch solution are added. 0.002 N permanganate is run in from a burette until the blue color of the iodide of starch appears. Using this procedure, 0.2 mg. of uric acid (the amount found in 20 cc. of blood) can be determined with an accuracy of 5 per cent or better. The results in blood, as well as the application of this new titration to other organic substances, are being studied further.

ALVEOLAR CARBON DIOXIDE OF THE NEW-BORN.

By MAX SEHAN.

METHODS OF DETERMINING HYDROGEN ION CONCENTRATION AND BUFFER VALUE (ALKALINE RESERVE).*

By J. F. McCLENDON.

THE ISOLATION AND IDENTIFICATION OF THE THYROID HORMONE.

By E. C. KENDALL.

A SIMPLE METHOD OF MAKING STALAGMOMETERS.

By A. D. HIRSCHFELDER.

THE FOOD VALUE OF SOME OF THE LESS USED PACIFIC COAST FISH.

By F. W. ALBRO and H. A. MATTILL.

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* Published in full in *J. Biol. Chem.*, 1918, xxxiii, 19

ABSTRACT OF THE EXECUTIVE PROCEEDINGS OF THE TWELFTH ANNUAL MEETING.

New Members: L. Jean Bogert, Florence H. Frankel, Frederick S. Hammett, Reuben L. Hill, Edward B. Meigs, Alice Rohde, Carl P. Sherwin.

Officers for the Year 1918: *President,* Carl L. Alsberg; *Vice-President,* Albert P. Mathews; *Secretary,* Stanley R. Benedict; *Treasurer,* Harold C. Bradley. *Additional Members of Council,* William J. Gies, Andrew Hunter, Elmer V. McCollum. *Nominating Committee,* Louis Baumann, W. R. Bloor, Philip B. Hawk, Walter Jones, V. C. Myers, J. R. Murlin, T. Brailsford Robertson, Frank P. Underhill, Donald D. Van Slyke.

Dues: The annual dues for the year 1918 were fixed at $2.00.

Stanley R. Benedict,
Secretary.

PROCEEDINGS

OF THE

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

THIRTEENTH ANNUAL MEETING

Baltimore, Md.

April 24–26, 1919

FOURTEENTH ANNUAL MEETING

Cincinnati, Ohio

December 29–31, 1919

ABSTRACTS OF PAPERS
ABSTRACT OF EXECUTIVE PROCEEDINGS

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For the List of Members and the Constitution of the Society see the Register of the Federation of American Societies for Experimental Biology comprising the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, and the American Society for Experimental Pathology.

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THE ETIOLOGY OF RICKETS.

By E. V. McCOLLUM, NINA SIMMONDS, and HELEN T. PARSONS.

(*From the Laboratory of Chemical Hygiene, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.*)

We have conducted an extensive series of experiments with rats restricted to diets derived from cereals and legume seeds; cereals, legume seeds, and muscle meats; and with similar diets in which degerminated products of cereal grains replaced whole seeds; and have supplemented these mixtures with purified food substances to determine the nature and extent of their dietary shortcomings.

In these experiments we have observed the gross picture of rickets in many of the animals restricted to faulty diets, and have demonstrated that this condition develops on diets in which the faults lie in several different factors.

A low content of fat-soluble A, low calcium content, poor quality of protein, and unsatisfactory salt combinations, acting in combinations, may all contribute to the etiology of the disease. We have not yet completed our observations on diets in which but a single factor is at fault. It is certain that specific fasting for fat-soluble A cannot be regarded as the sole and only possible cause of rickets.

Since the same gross picture can be induced in several different ways, we are led to suggest the possible occurrence of more than one kind of rickets. Histological studies of tissues of animals suffering from what appears to be rickets, but from different causes, are still in progress. No decision can yet be reached as to whether in all cases the histological picture is the same in animals exhibiting beaded ribs, enlargements of the costochondral junctions, deformity of the thorax, and general deformity of the body, irrespective of the dietary factor or factors which brought about the condition.

THE RÔLE OF FAT-SOLUBLE VITAMINE IN HUMAN NUTRITION AND ITS SUGGESTED RELATION TO RICKETS.

By ALFRED F. HESS.

(*From the Bureau of Laboratories, Department of Health, New York.*)

It has been shown that the fat-soluble vitamine is an essential constituent of the dietary of rats. There have also been clinical reports attributing marked malnutrition in infants and children to a lack of this dietary factor (Japan, Denmark). As a result of these experiences it has been accepted that this vitamine is highly important for man, and that the lack of it leads to nutritional disorder in children. This has been emphasized all the more as this vitamine is not nearly so widely distributed in nature as is the water-soluble vitamine. In order to study this question five infants, varying in age from 5 to 12 months, were given a diet which was complete except for a very small amount of fat-soluble vitamine. It consisted of 180 gm. daily of highly skimmed milk ("Krystalak" 0.2 per cent fat), 30 gm. of cane sugar, 15 to 30 gm. of autolyzed yeast (to supply water-soluble vitamine), 15 cc. of orange juice, 30 gm. of cottonseed oil, and cereal for the older infants.

On this diet the children have done well for a period of 8 to 9 months. They have shown no anemia, no eye trouble, no bone changes, as seen by the x-ray, nor has their growth in length or in weight suffered. We believe, therefore, that either a very small amount of this vitamine suffices to supply the needs of human nutrition, or that this deficiency has to be maintained for a period of years in order to bring about any harmful result. Danger from a lack of this dietary factor need not be apprehended if the diet is otherwise complete.

The development of rickets has been attributed by Mellanby, as a result of experiments on dogs, to a lack of fat-soluble vitamine, and Hopkins and Chick have termed this vitamine the "anti-rachitic factor." It was found, however, that infants fed on this "fat-soluble vitamine minimal diet" did not develop the well established signs of rickets—beading of the ribs, enlargement of the epiphyses, weakness of the muscles, etc. We cannot believe, therefore, that rickets is brought about merely by a deficiency of this principle; all the more so, as this disorder developed in infants receiving large quantities of milk containing ample fat-soluble vitamine. It may be added that neither cream nor the leafy vegetables, both of which are rich in this principle, are comparable to cod liver oil as growth stimulants.

PRELIMINARY OBSERVATIONS ON THE RELATION OF BACTERIA TO EXPERIMENTAL SCURVY IN GUINEA PIGS.

BY MAURICE H. GIVENS AND GEORGE L. HOFFMANN.

(*From the Research Laboratories, Western Pennsylvania Hospital, Pittsburgh.*)

Whether or not bacteria play any rôle in the development of scurvy in guinea pigs has not been settled by direct evidence. Jackson and Moore[1] found coccus-like bodies in microscopic sections of lesions in scorbutic guinea pigs. Jackson and Moody[2] isolated from the diseased joints, muscles, and lymph glands of these animals Gram-positive and Gram-negative organisms. Pure strains of these bacteria introduced into guinea pigs gave rise in most instances to hemorrhagic and other lesions in the bones, joints, muscles, lymph glands, and organs. Torrey and Hess[3] concluded that scurvy, both of guinea pigs and of infants, was not associated with an overgrowth of putrefactive bacteria in the intestinal tract.

We have attempted to throw further light upon the question by bacteriological examinations of the blood, joints, and feces of guinea pigs made scorbutic on different diets and then treated with different antiscorbutic foods. Blood from scorbutic animals anesthetized and from those dying of the disease regardless of the diet producing the same has been found to be sterile. The enlarged front joints of guinea pigs developing scurvy on oats alone were sterile; this was likewise true in the majority of cases of guinea pigs developing scurvy on the soy cake food of Givens and Cohen.[4] However, in two or three instances a staphylococcus and diplococcus were isolated. Pure strains of these organisms injected intracardially, intraperitoneally, and into the joints of healthy guinea pigs on a mixed diet produced no signs of scurvy. Smears and cultures were made of material from different parts of the intestinal tract of guinea pigs on oats alone, on oats plus lemon juice, 3 cc. daily, after scurvy developed, on the soy cake diet, and on the same plus cabbage after the appearance of scurvy. No marked difference was found in the intestinal flora under any of these conditions.

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[1] Jackson, L., and Moore, J. J., *J. Infect. Dis.*, 1916, xix, 510.

[2] Jackson, L., and Moody, A. M., *J. Infect. Dis.*, 1916, xix, 511.

[3] Torrey, J. C., and Hess, A. F., *Proc. Soc. Exp. Biol. and Med.*, 1917–18, xv, 74.

[4] Givens, M. H., and Cohen, B., *J. Biol. Chem.*, 1918, xxxvi, 127.

FURTHER STUDIES ON THE USE OF WATER-SOLUBLE B IN THE TREATMENT OF INFANT MALNUTRITION.

By WALTER H. EDDY.

(*From the Society of the New York Hospital, New York.*)

Results of experiments were reported confirming previous work[5] of the author in stimulating growth by the addition of B vitamine extract to the diet of infants suffering from malnutrition of the marasmus type. A new feature used in the study was the application of the Bachmann test[6] to measurement of dosage.

In experiments with vitamine prepared from the navy bean by the McCollum method[7] the test detected relatively small amounts of vitamine and, while in need of further standardization, offered a valuable aid in measurement of the vitamine B present in the substances used. Tables were shown giving the result of the test on various amounts of the dextrin-vitamine mixture and on other substances such as milk, both cow and human milk.

The first case, showing stimulation with the B vitamine, gained an average of 0.84 ounces per day in a 32 day period as against a gain of 0.47 ounces per day during a 17 day period preceding the use of the vitamine through the calorie intake and the food given remained constant through both periods. The second case showed a similar stimulation though not so well controlled as the first. The interesting feature of the use of the Bachmann test as applied to the first case was the result of the tests as applied to the child's diet and to the extract. The diet was found to contain 2,120 units of vitamine and the stimulating mixture only 70 units.

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[5] Eddy, W. H., and Roper, J. C., *Am. J. Dis. Child.*, 1917, xiv, 189.

[6] Bachmann, F. M., *J. Biol. Chem.*, 1919, xxxix, 235.

[7] McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, 1918, xxxiii, 55.

In other words an increase of only 3 per cent in actual vitamine intake produced the marked stimulation. The author suggested that this result may be due to the fact that the child could utilize the extracted vitamine when it could not utilize the vitamine in the diet and that the way the vitamine is held in a diet may be an important factor. In all the baby cases treated the extract feeding is followed by an increased growth which continued to a point where removal of the extract is possible without marked reduction in the growth rate and the child then goes on to recovery. These cases represent the fifteenth and sixteenth showing stimulation under this treatment.

THE EXOGENOUS AND ENDOGENOUS NATURE OF THE CREATINURIA IN THE GROWING DOG.

By VICTOR JOHN HARDING and ELRID G. YOUNG.

(*From the Biochemical Laboratory, McGill University, Montreal.*)

In investigating the creatinuria of pups, and studying the effect of high and low protein diets upon that condition, the authors find in most of the animals that the creatine excretion is dependent upon the level of protein intake. Only in one animal, however, did they find that the creatinuria could be totally abolished by a low protein diet, and even in this case creatine again made its appearance in the urine in spite of the low protein diet. In most of the animals investigated there always remained a residuum of creatine in the urine, which invariably increased on a continuation of the low protein diet. This increase in the creatine excretion, following its lowering with a low protein diet, is ascribed to the production of that substance from some store of intermediary metabolites. The authors are thus of the opinion that creatine can be of both exogenous and endogenous origin and that both can exist in the same animal. The endogenous creatine is not a constant quantity, and its amount is determined by factors as yet unknown. In two animals, however, the authors were unable to affect the excretion of creatine by an alteration in the protein of the diet. In these animals apparently the endogenous fraction is so large as to obscure the exogenous portion.

CYSTINE AS THE PRECURSOR OF CREATINE IN THE GROWING DOG.

By VICTOR JOHN HARDING and ELRID G. YOUNG.

(*From the Biochemical Laboratory, McGill University, Montreal.*)

The authors have investigated the effects upon the exogenous creatine excretion in pups of variations in the amino-acid content of the protein.

Arginine is without any effect. A variation in the content of the cystine in the protein or the addition and withdrawal of that amino-acid as such is followed by a variation in the creatine excretion. It is found, however, that as well as affecting the exogenous function of the creatine the endogenous portion is also disturbed.

The hypothesis has been formulated that cystine forms creatine through the intermediate stages of taurine and amino ethyl alcohol, followed by methylation, combination with urea, and oxidation.

THE ORIGIN OF CREATINE.

By R. B. GIBSON and FRANCES T. MARTIN.

(*From the Chemical Research Laboratory, Department of Theory and Practice of Medicine, the State University of Iowa, Iowa City.*)

Ingested creatine is promptly eliminated chiefly as creatine, in part as creatinine, in cases of advanced progressive pseudohypertrophic muscular dystrophy. The creatine and to a lesser extent the creatinine are increased with a high protein intake inasmuch as in the case reported half again as much creatinine and twice as much creatine may be excreted as on a low nitrogen plane. This increase is obtained from the catabolized protein (including gelatin) and not from that retained for growth purposes. The substitution of the arginine-rich protein edestin for the protein of the diet does not affect the creatine output. Ingested asparagine and sarcosine do not lead to increased creatine formation; glycocyamine is converted in part (at least 36 per cent) in a controlled experiment, thus confirming earlier work. It is probably not a stage in the ordinary creatine formation.

CORRELATION OF CERTAIN PHYSICAL AND CHEMICAL FACTORS WITH TOXICITY TO MARINE ORGANISMS.

By G. H. A. CLOWES and L. G. KEITH.

(*From the Biochemical Research Laboratory, Eli Lilly and Company, Indianapolis.*)

The absolute and relative toxicities of the symmetrical and asymmetrical dichloroacetones to developing sea urchin eggs correspond with their toxicities to mice, the symmetrical compound being sixty times as toxic as the asymmetrical.

Experiments with mustard gas and other war gases have indicated that their relative toxicities depend upon rate of hydrolysis, lipoid water distribution coefficient, vapor tension, etc. The dichloroacetone experiments support this view. The symmetrical compound diffuses more rapidly from a non-aqueous to an aqueous phase, and hydrolyzes more rapidly in a feebly alkaline aqueous phase than does the asymmetrical.

With both substances a latent period was observed, during which the gases could be removed by shaking with additional eggs or charcoal, the protective effect depending upon temperature, time of application, and amount of protective substance employed.

An analysis of the data obtained by exposing varying numbers of eggs to varying concentrations for varying times at varying temperatures, and subjecting them to varying protective procedures, supports the view previously advanced that the war gases are first adsorbed on the surface of the protoplasmic structure, then penetrate through protoplasm by diffusion, and finally after a latent period undergo hydrolysis causing the death of the cell.

METABOLISM OF O-NITROBENZALDEHYDE, M-NITROBENZALDEHYDE, AND P-NITROPHENYLACETALDEHYDE.

By CARL P. SHERWIN, JOHN A. DALY, and WALTER A. HYNES.

(*From the Research Laboratory, Fordham University, New York.*)

According to the investigations of Cohn, *o*-nitrobenzaldehyde, when fed to the rabbit, is mostly destroyed, and only about 10 per cent is excreted in the urine as *o*-nitrobenzoic acid. *m*-Nitrobenzaldehyde is excreted by the rabbit as *m*-acetylaminobenzoic acid, and by the dog as *m*-nitrohippuric acid urea. *p*-Nitrobenzaldehyde is excreted by the rabbit as a combination of *p*-nitrobenzoic acid and *p*-acetylaminobenzoic acid, and by the dog *p*-nitrobenzaldehyde is excreted in the urine as *p*-nitrohippuric acid urea.

We fed 2 gm. of *o*-nitrobenzaldehyde to a man and recovered 65 to 70 per cent of *o*-nitrobenzoic acid from the urine. No reduction of the nitro group was apparent in this case, nor was there any combination with glycocoll by the *o*-nitrobenzoic acid thus produced.

m-Nitrobenzaldehyde was fed to a man in 5 and 6 gm. doses. From the urine we recovered about 75 per cent of the substance in the form of *m*-nitrobenzoic acid, and after the 6 gm. dose a fraction of a gm., about 5 per cent, was found as *m*-nitrohippuric acid.

p-Nitrophenylacetaldehyde was fed to a rabbit in 1 gm. doses, and 75 to 80 per cent was excreted as *p*-nitrophenylacetic acid, while *p*-nitrophenylacetic acid, when fed, resulted in rapid elimination of the same substance in the urine. 5 gm. of *p*-nitrophenylacetaldehyde were also fed to a dog and excreted in the urine as *p*-nitrophenylacetic acid. *p*-Nitrophenylacetic acid, as previously shown by one of us, is excreted mostly as the same substance in the urine of the dog, but a small fraction was also excreted in combination with glycocoll as *p*-nitrophenaceturic acid. 5 gm. of *p*-nitrophenylacetaldehyde were ingested by a man and 70 per cent of this was recovered from the urine as *p*-nitrophenylacetic acid. *p*-Nitrophenylacetic acid, as we have previously shown, is excreted by the human uncombined.

In these cases we could find no reduction of the nitro group in any of the aldehydes, as found by Cohn in his work on rabbits, so we fed 5 gm. of *p*-aminophenylacetic acid to a human, and again to a dog. In both cases there appeared in the urine a dark red substance, easily soluble in all organic solvents and insoluble in water. This substance has not as yet been crystallized or identified.

RELATION BETWEEN AMMONIA EXCRETION AND THE HYDROGEN ION CONCENTRATION OF URINE.

By CYRUS H. FISKE.

(*From the Biochemical Laboratory, Harvard Medical School, Boston.*)

Hasselbach's supposed constant relation between the ammonia coefficient and the C_H of urine is subject to deviations of sufficient frequency to suggest that other controlling factors are concerned. An examination has consequently been made, on hourly samples of human urine, of (1) the C_H, and (2) a ratio, that is more likely than the ammonia coefficient to have a real significance in connection with the regulation of reaction; *viz.*, the ratio of ammonia to sulfuric acid. Other things being equal, this ratio rises with the C_H, but the relation is by no means constant under all conditions. At the same C_H, the ratio is lower the higher the sulfate content, and also the higher the phosphate content. The dependence on the sulfate excretion may be explained by the fact that acids, other than sulfuric, that call for the formation of ammonia, are relatively less in amount the greater the rate of production of sulfuric acid, while the dependence on the phosphate output is perhaps to be accounted for by the supposition that the demand for ammonia is less the greater the amount of phosphate available for excretion, since the conversion of secondary to primary phosphate liberates fixed alkali.

THE AMINO-ACID CONTENT OF BLOOD FROM THE JUGULAR AND MAMMARY VEINS OF MILKING COWS.

By C. A. CARY.

(*From the Research Laboratories of the Dairy Division, Bureau of Animal Industry, United States Department of Agriculture.*)

An attempt was made to determine the change in the amino-acid content of blood and of blood plasma as it passes through the mammary gland of milking cows by comparing the amino-acid N in samples obtained as nearly simultaneously and with as little disturbance as practical from the jugular and mammary (abdominal subcutaneous) veins.

The proteins were removed by the coagulation-trichloroacetic acid-kaolin method described by Bock,[8] the amino N being determined by the HNO_2 method, using the smaller apparatus of Van Slyke. The urea, etc. were corrected for by making duplicate runs allowing a longer time for deamination.[9]

The averages of duplicate analyses of plasma are given in Table I.

TABLE I.

Vein.	Amino-acid content of 100 cc. of blood plasma.					
	I	II	III	IV	V	VI
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Jugular	2.68	3.31	2.41	2.49	2.38	2.47
Mammary	1.78	2.34	2.45	1.93	2.00	1.87
Difference	0.90	0.97	0.04	0.56	0.38	0.60

The cows gave about 10 kilos of milk daily. The plasma constituted about 67 per cent by volume of the whole blood. Using a very rough approximation[10] of the flow of blood through the mammary gland, assuming that the difference in amino-acid content of the mixed arterial and jugular bloods is relatively negligible, and that the changes in the plasma thus indicated are effected continuously throughout the 24 hours, our largest differences indicate that about 35 gm. of amino-acid N are abstracted daily from the plasma passing through the gland. This estimate is rough and it is very doubtful whether we get samples of blood indicating the maximum changes occurring in the gland. It is, however, suggestive relative to the formation of milk proteins.

With the whole blood the differences are in general in the same direction as those found in the corresponding plasma, but more work must be done to determine whether there is any change or not in the amino-acid N of the corpuscles of blood perfusing the gland. The work is still in progress.

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[8] Bock, J. C., *J. Biol. Chem.*, 1916–17, xxviii, 357.

[9] Van Slyke, D. D., and Meyer, G. M., *J. Biol. Chem.*, 1912, xii, 399.

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NEUTRALITY REGULATION IN CATTLE.

By N. R. BLATHERWICK.

(*From the Research Laboratories of the Dairy Division, Bureau of Animal Industry, United States Department of Agriculture.*)

The alkaline reserve of the plasma of cows, as measured by the CO_2 capacity, is remarkably constant. The average normal value, 60.9 cc. of CO_2 per 100 cc. of plasma, is somewhat lower than that of normal human plasma. Maximum and minimum values were 65.5 and 55.1 cc., respectively. The CO_2 capacity of the plasma of three pregnant cows showed no evidence of the acidosis of pregnancy observed by others in women. By feeding a cow on rations restricted to one food, namely hay, grain, or silage, changes in plasma CO_2 capacity and in the ammonia and CO_2 of the urine were produced. A cow fasted for 7 days failed to show a decrease in the CO_2 capacity of the plasma, but rather a slight increase apparent at 24 hours, which was maintained throughout the fast. Coincident with this was an increase of 11 per cent in the inorganic P of the plasma. Larger increases in the inorganic P of the plasma, as the result of fasting, have been found in this laboratory. This change indicates a mobilization of bone substance to assist in the maintenance of neutrality. Another important factor in maintaining the correct reaction of the cow's body is the excretion of CO_2 in the urine. This has been observed to vary from 6 cc. per 100 cc. in the urine of a young calf living on a milk diet to 394 cc. in that of a cow eating silage, grain, and hay.

EFFECT OF ACIDS, ALKALIES, AND SALTS UPON CATALASE PRODUCTION.

By W. E. BURGE.

(*From the Department of Physiology, University of Illinois, Urbana.*)

100 cc. of 0.15 per cent hydrochloric acid decrease the catalase of the blood about 30 per cent in 2 hours, while a similar quantity of acetic acid increases it about 14 per cent. The decrease produced by the hydrochloric acid is due in part to the destruction of the enzyme and in part to the inhibiting action of the acid; the increase produced by the acetic acid is due to the stimulation

of the alimentary glands, particularly the liver, to an increased output of the enzyme. Carbonic acid was found to have no effect on the catalase of the blood. 10 gm. per kilo of sodium carbonate as well as sodium acetate dissolved in 100 cc. of water increase the catalase of the blood. The animals used in these experiments were rabbits and the substances were introduced by means of a stomach tube.

A STUDY OF THE DISTRIBUTION OF CATALASE IN THE KIDNEY.

By SERGIUS MORGULIS and VICTOR E. LEVINE.

(*From the Department of Biochemistry and Physiology, College of Medicine, Creighton University, Omaha.*)

Experiments with the kidney of rabbit, sheep, pig, and beef, perfused with saline and unperfused, indicate a catalase content (quantity of hydrogen peroxide decomposed) and a catalytic activity (rate of decomposition) which are much greater in the cortex than in the medulla. The upper portion of the medulla yields greater values than the papillary portion. Part of the catalase is derived from the blood and can be removed by perfusion; part remains insoluble even after prolonged perfusion. Results with the right and the left kidney from the same animal point to the fact that the two organs differ in catalase content and catalytic activity. The kidney tissue contains no direct oxidizing enzyme. Quantitative experiments on peroxidase show that the peroxidase relations are the opposite of those of catalase. The greatest peroxidase content and peroxidase activity (rate of reaction) are found in the medulla; the cortex values are comparatively small. Perfusing the kidney results in the complete removal of peroxidase, although catalase is removed only in part by this procedure.

THE SIGNIFICANCE OF CONCENTRATION WITH REFERENCE TO SUBSTANCES IN THE BLOOD PLASMA.

By R. T. WOODYATT.

(From the Laboratory of Clinical Research, Rush Medical College, the Otho S. A. Sprague Memorial Institute, Chicago.)

A dog weighing 10 kilos received glucose by vein constantly for 8 hours at the uniform rate of 10 gm. per hour. In the first 4 hours the glucose was given in dilute solution; in the second 4 hours in concentrated solution; *i.e.*, first in about 3 and later in about 36 per cent form. The dog passed in the urine 0.1 to 0.2 gm. of sugar per hour throughout (except in the first hour). In the 2nd, 3rd, 4th, and 5th hours the blood sugar percentage was 0.13 to 0.14 per cent. In the 6th, 7th, and 8th hours it was 0.21 to 0.23 per cent. This change followed the change in the volume of water injected and occurred while the rates of glucose injection, glucose elimination, and hence glucose utilization all remained constant. If anything, the glucose excretion was a trifle higher during the period of low blood sugar percentages. The experiment is one of a group and depicts a type.

The phenomenon was discussed in relation to the observations of Magnus on salts; and Epstein on the blood sugar and rate of glycosuria in diabetes. It was pointed out that if the surface of contact between the blood and the cells should vary in direct proportion to the blood volume, the results would be explained. Anatomical details of capillary systems were discussed to show how an increasing volume of capillary blood—especially by opening up erstwhile empty capillary collaterals—may develop surface approximately in proportion to the volume of extra blood.

In interpreting the physiological effects of a substance in the blood plasma, the factor of concentration alone is not sufficient. Surface is also important. Possibly during normal variations of the blood volume, surface and volume vary in direct proportion.

ALKALOID DIFFUSION IN PHYSICAL AND BIOLOGICAL SYSTEMS.

By G. H. A. CLOWES and A. L. WALTERS.

(From the Biochemical Research Laboratory, Eli Lilly and Company, Indianapolis.)

Alkaloids adsorbed by fullers' earth cannot be released by extraction with water or an aqueous solution of sodium bicarbonate having the alkalinity of intestinal contents. Nevertheless when the alkaloid adsorption compound is administered by mouth, the alkaloid is adsorbed by the intestinal mucosa. It was found, for example, that adsorbed atropine administered to cats by mouth produced a prolonged dilatation of the pupil of the eye. Adsorbed strychnine caused convulsions and death in rabbits and rats, and adsorbed quinine and emetine were recoverable in the urine. Adsorbed strychnine introduced into the peritoneal cavity caused convulsions and death, but when the adsorbed strychnine was first introduced into a collodion sac which was freely permeable for strychnine but would not permit of the passage of cells, and the sac introduced into the peritoneal cavity, there was no effect.

These experiments show that the alkaloid may be extracted by protoplasm in a neutral medium and suggest the probability that the alkaloid is released by direct contact between the protoplasmic material of the cell and the adsorption compound.

In the above experiments from two to ten times the amount of adsorbed alkaloid was required to produce a result equivalent to that obtained in control experiments with free alkaloid. Alkaloids were extracted from their adsorption compounds to a slight extent by intestinal contents, somewhat better by means of intestinal mucosa, egg yolk, etc., but with considerable facility by so called neutral soaps and soaps to which either fatty acid or alkali had been added. The extracts were carried out either in aqueous or mixed aqueous organic media or in emulsions using soaps of sodium, potassium, calcium, and magnesium.

These results support the view that soaps and the fatty acid group play an important rôle in protoplasmic assimilation, and explain the therapeutic effects obtained by administering alkaloids in the adsorbed form.

THE NATURE OF THE LIGHT-PRODUCING REACTION OF LUMINOUS ANIMALS.

By E. NEWTON HARVEY.

(From the Physiological Laboratory, Princeton University, Princeton.)

Animal luminescence results from the oxidation of a substance, luciferin (LH_2), in presence of a catalyst, luciferase, water, and oxygen. When solutions of luciferin and luciferase are mixed, not enough CO_2 is produced to saturate the buffers in these solutions and probably no CO_2 at all is produced; not enough heat is produced to raise the temperature of the mixture 0.001°C., and from this figure the heat of oxidation of luciferin is calculated to be less than 0.1 calorie per gm. The oxidation product of luciferin, oxyluciferin (L), is very similar to luciferin and may be reduced to luciferin again by various perhydridases and by nascent hydrogen or H_2S. The reduction is accelerated by acid and in the presence of light. Thus the reaction is photogenic in one direction, photochemical in the opposite direction, and may be represented as follows:

$$\text{Luciferin } (LH_2) + O \underset{\substack{\text{Perhydridase or nascent H.} \\ \text{Acid.} \\ \text{Light.}}}{\overset{\substack{\text{Darkness.} \\ \text{Alkali.} \\ \text{Luciferase.}}}{\rightleftarrows}} \text{oxyluciferin } (L) + H_2O$$

THE INFLUENCE OF OXYGEN DEFICIENCY AND RELATED CONDITIONS UPON THE HEMATO-RESPIRATORY FUNCTIONS.

By YANDELL HENDERSON and HOWARD W. HAGGARD.

(From the Physiological Laboratory, School of Medicine, Yale University, New Haven.)

It is generally supposed that under oxygen deficiency acids are produced in the tissues and are retained in the blood, and that the blood alkali is thus neutralized and eliminated through the urine. This may be termed the acidotic process.

Experiments on dogs subjected to progressively decreasing oxygen show, however, that the process actually involved is in many respects exactly the opposite of the usual supposition. They demonstrate that before any considerable amount of alkali is lost an abnormally large amount of CO_2 is eliminated by the excessive breathing induced by a lowered oxygen pressure in the air breathed. Then alkali passes out of the blood to compensate this alkalosis. This we term the acapnial process.

From these facts and related observations on men it appears that in normal persons the blood alkali is controlled by the dissolved CO_2—more or less alkali being called into use in the blood to satisfy the normal $H_2CO_3 : NaHCO_3$ relation, and thus to keep the C_H of the blood nearly constant. The amount of dissolved CO_2 in the blood is controlled by the pulmonary ventilation, and fundamentally in normal persons the ventilation is adjusted to the oxygen partial pressure of the altitude at which the person lives.

When, however, overbreathing is induced and the H_2CO_3 of the blood is reduced, the $NaHCO_3$ follows downward. This, we find, and not acidosis, is what occurs also in carbon monoxide asphyxia.

A simple test for differentiating between low blood alkali of acidotic and that of acapnial origin is the administration of air to which 8 to 10 per cent of CO_2 has been added. This quickly causes death in acidotic subjects; but it induces a rapid recall of alkali to the blood, and restoration of the subject to a virtually normal condition of health if the condition is of acapnial origin.

We have now (in collaboration with Dr. R. C. Coburn) applied this treatment to patients after surgical operation and anesthesia. The result has been that alkali was recalled to the blood, that arterial pressure and other functions were restored to normal, and that the anesthetic was rapidly eliminated by the full breathing during the CO_2 inhalation with a consequent great reduction of nausea.

In dogs asphyxiated with carbon monoxide (in investigations for the United States Bureau of Mines) strikingly beneficial results have been obtained by means of oxygen reenforced with 10 per cent CO_2.

RELATION OF ANESTHESIA TO RESPIRATION.

By SHIRO TASHIRO.

(From the Biochemical Laboratory, College of Medicine, University of Cincinnati, Cincinnati.)

The rate of the tissue respiration of the claw nerves of various crabs is highest in lobster, then spider crab, and least in *Limulus*. That of the sciatic nerve of frog comes between the last two. The time required for complete anesthesia with the same narcotics is shortest for lobster, then spider crab, frog, and *Limulus* in order. Susceptibility of the nerve to anesthetics runs parallel to the rate of its respiration, provided that other factors are fairly constant. A 0.4 per cent solution of chloral hydrate does not alter the original gradient of respiration of the spider crab's nerve, but 2 per cent, which produces reversible loss of irritability, reverses it. These results can be best explained if we accept the idea that susceptibility of the same nerve toward anesthetics is quite different along the fiber, depending mainly upon the different respiratory activities of the different parts of the fiber. The advantage of using tissue with as few functions as possible is obvious, for we must know exact physiological conditions of all parts of the tissue when we measure the respiration. Abolition of one function in a tissue may or may not mean complete anesthesia of the tissue as a whole. It is suggested that primary stimulation by weak concentrations of narcotics is probably caused by exaggerating the original metabolic gradient; and final anesthesia, by further oxidative interference which primarily changes the relation between tissue respirations of the different parts of the tissue.

DETERMINATION OF METHEMOGLOBIN IN BLOOD.

By W. S. McELLROY.

(From the Laboratory of Physiological Chemistry, School of Medicine, University of Pittsburgh, Pittsburgh.)

When methemoglobin is present the colorimetric determination of hemoglobin cannot be used as an index of the oxygen-carrying capacity of the blood. Under these conditions the oxygen capacity must be determined directly.

Methemoglobin interferes with the colorimetric determination of hemoglobin as carbon monoxide hemoglobin and acid hematin.

The total hemoglobin in the presence of methemoglobin can be determined colorimetically by converting the oxyhemoglobin into methemoglobin by means of potassium ferricyanide or other suitable reagent and determining the total as methemoglobin.

The oxygen capacity gives the amount of oxyhemoglobin.

The difference between the total hemoglobin determined as methemoglobin and the oxyhemoglobin estimated from the oxygen capacity gives the amount of methemoglobin.

BLOOD PHOSPHATES IN THE LIPEMIA PRODUCED BY SEVERE HEMORRHAGE.

By W. R. BLOOR and E. D. FARRINGTON.

(From the Department of Biochemistry and Pharmacology, University of California, Berkeley.)

Changes directly connected with the lipemia were confined to increases in lipoid phosphorus in plasma (up to five times the normal value) and in corpuscles (double the normal value).

Changes attributable to the bleeding and regeneration were mainly increases in the inorganic phosphates in the corpuscles and, to a less extent, changes in the quantities of the other phosphoric acid combinations.

FAT CONTENT OF EMBRYONIC LIVERS.

By C. G. IMRIE and S. G. GRAHAM.

(From the Laboratory of Pathological Chemistry, University of Toronto, Toronto.)

Observations were made upon the fat content of the livers of embryonic guinea pigs throughout the period of gestation. Such changes, if any, had first to be established before the relation of the embryonic liver to fat mobilized from the storehouses in the pregnant animal could be interpreted. The results of this preliminary investigation were reported. The higher fatty acid content was determined by saponification and extraction, as described by Leathes, and the iodine value by the method of Wij. The weight of the embryo was employed as an indication of its age. Until the embryo reaches a stage in its development corresponding to a weight of 35 to 40 gm., the fat content of the liver more or less closely approximates that of the maternal liver, which is from 2 to 3 per cent. After this period, however, there is a progressive accumulation of fat in the embryonic liver, so that at birth the animal has from 16 to 18 per cent of higher fatty acids reckoned upon the moist tissue. The iodine value of this fat was comparatively high, though lower than that in the maternal liver.

A study of the fate of this fat showed that it is rapidly utilized by the animal during the first 48 to 72 hours of its life. As the fat content is lowered during this period, the iodine value rises.

Figures and charts representing these changes were presented.

QUANTITATIVE COLORIMETRIC DETERMINATION OF TYROSINE AND TYRAMINE (P-HYDROXYPHENYLETHYLAMINE) AND OTHER PHENOLS.

By MILTON T. HANKE and KARL K. KOESSLER.

(*From the Otho S. A. Sprague Memorial Institute and the Departments of Pathology and Physiological Chemistry, University of Chicago, Chicago.*)

A method has been developed for estimating tyrosine, tyramine *p*-cresol, oxyphenylacetic acid, and phenol by means of which quantities as small as 0.000005 gm. of these substances can be determined. The method is based upon the interaction, in alkaline solution, of the phenols with *p*-phenyldiazonium sulfonate. The color so obtained is intensified and stabilized by the addition of NaOH and hydroxylamine hydrochloride. The theory of the reaction involving the stabilization of the quinone ring by the hydroxylamine was discussed in detail.

QUANTITATIVE COLORIMETRIC DETERMINATION OF HISTIDINE AND HISTAMINE IN PROTEINS AND PROTEIN-CONTAINING MATTER.

BY KARL K. KOESSLER AND MILTON T. HANKE.

(From the Otho S. A. Sprague Memorial Institute and the Departments of Pathology and Physiological Chemistry, University of Chicago, Chicago.)

A method has been developed for estimating histidine and histamine in proteins and protein-containing matter that is based upon the method developed for pure imidazoles and previously described by us,[11] and upon the method developed by Van Slyke for the estimation of the hexone bases.[12]

DIGESTIBILITY OF RAW CORN-STARCH.

BY C. F. LANGWORTHY AND HARRY J. DEUEL, JR.

(From the Office of Home Economics, States Relation Service, United States Department of Agriculture.)

Raw starches are generally considered to be digested only slightly, if at all, by the human body. In the experiments here reported the digestibility of raw corn-starch when eaten in quantity by normal men was determined. There were some variations, but on an average the three subjects ate 250 gm. of raw corn-starch a day during the 3 day experimental period, in the form of a frozen custard, in which the starch was combined with milk, sugar, and a little salt, and flavored with lemon extract to mask the "starchy" taste. The methods were those usually followed in experiments with cereals carried on in this laboratory. The total amount of feces pertaining to the experimental period was very small and on examination did not show any starch.

The digestibility of the raw corn-starch was found to be 100 per cent. So far as could be seen, its ingestion had no abnormal physiological effects and the subjects remained in their accustomed good health.

The experiments here reported are the first of a series dealing with the digestibility of raw starches, in which it is planned to include potato, rice, wheat, arrowroot, and possibly other starches.

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[11] Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 1919, xxxix, 521.

[12] Van Slyke, D. D., *J. Biol. Chem.*, 1911–12, x, 15.

SPERM AS AN INHIBITANT OF MATURATION AND FERTILIZATION.

By G. H. A. CLOWES and ESTHER GREISHEIMER.

(From the Biochemical Research Laboratory, Eli Lilly and Company, Indianapolis.)

Immature starfish eggs are prevented from maturating by treatment with butyric acid, exposure to heat, etc., the identical procedures which, after maturation, induce artificial parthenogenesis.

Similarly exposure of the immature eggs to sperm, even in small amounts, inhibits or retards maturation rendering the eggs unfertilizable or difficultly fertilizable by sperm, at a subsequent stage at which control eggs not previously treated with sperm are readily fertilized.

The best contrasts may be obtained by giving the immature eggs a preliminary exposure to a weak butyric acid solution which retards but does not prevent maturation. This treatment followed immediately by exposure to sperm causes a marked interference with maturation and subsequent fertilization as compared with eggs which receive the butyric treatment only. Heat followed by sperm gives similar results.

Inhibiting effects of sperm on immature eggs may be counteracted by a brief treatment with 0.001 N $NaOH$, but not ammonia. The similarity of inhibiting effect obtained by butyric acid and sperm separately or in combination, and the reversal of these effects by $NaOH$, suggest that inhibiting effect of sperm is attributable to production of acid on or adjacent to egg surface.

COLLOID CHEMISTRY OF SOAPS AND PROTEINS.

By MARTIN H. FISCHER.

(From the Physiological Laboratory, University of Cincinnati, Cincinnati.)

On the assumption that the polymerized amino-acids which we call proteins react with bases of various sorts to form salts in the same fashion in which fatty acids unite with bases of various sorts to form soaps, the colloid chemistry of the latter was studied to obtain light regarding the colloid behavior of the former.

The hydration capacity of the soaps varies with the basic radical and, in general, in the order: NH_4, K, Na, Li, Mg, Ca, Fe, Cu, Hg. It varies also with the type of fatty acid, that standing lowest in any homologous series being least hydratable. When different series are compared, the less saturated possess the lower hydration capacity.

Colloid soap jellies may be prepared from the various soaps and anhydrous solvents, like monatomic, diatomic, and triatomic alcohols, aldehydes, benzene, toluene, etc. This finding speaks against the too heavy emphasis now being placed upon purely electrical notions of stabilization in colloid systems.

Hydrophilic or lyophilic colloids are defined as dispersions, coarser than molecular, of one material in a second with the dispersed substance a solvent for the dispersion medium.

Liquid soaps may be "coagulated" by alkalies and neutral salts of various kinds even when chemical reaction between soap and added substances is impossible. The consecutive changes of setting, secondary liquefaction with progressive dehydration of the soap, and its separation in "coagulated" form are explained on the basis of union between added salt and water with production of an emulsion of salt water in soap succeeded by one of soap in salt water and ending in anhydrous soap floating upon salt water.

Chemically neutral soaps do not affect an indicator like phenolphthalein in concentrated solution. On diluting with water they turn it bright red. This means either (1) that water-in-colloid systems (analogous to normal cells and body fluids) cannot be properly analyzed by indicator methods but only colloid-in-water systems (analogous to the secretions from the body) or (2) that the former contain no ions.

From qualitative and quantitative analogy in colloid behavior between soaps and protoplasm the latter is held, in essence, to be a salt compound of various polymerized amino-acids with potassium, sodium, magnesium, calcium, etc., which materials have "dissolved" a certain amount of water. Introduction of acid, ammonia, or potassium into this compound represents poisoning by these materials at one end of such a soap-like series (as evidenced by increased hydration capacity and increased solubility in water); introduction of the heavy metals poisoning at the other end (as evidenced by a decreased hydration capacity and the production of difficultly soluble compounds). Just as a soap anywhere in the series can, by appropriate means, be converted into any other, just so can a normal protein or living cell be "poisoned" through the addition of materials found at either extreme in the base series; or after such poisoning be changed to nearer the normal by addition of the bases in the middle series.

PATHOGENESIS DUE TO VITAMINE DEFICIENCY IN THE RAT.

BY A. D. EMMETT AND FLOYD P. ALLEN.

(*From the Research Laboratory of Parke, Davis and Company, Detroit.*)

The rats were fed on definite dietary planes which would produce normal growth except for the lack of either vitamine A or B. From these groups, animals were selected which were representative. Care was taken to see that the animals were free from infection. Control tests were made on tissues from rats fed a complete diet.

The various tissues examined histologically were: thymus, thyroid, pancreas, testes, ovaries, adrenals, liver, spleen, ileum, colon, kidneys, heart, lungs, brain, optics, lower cord, and sciatic nerves. In the absence of vitamine B, the most noticeable changes were found in the almost complete atrophy of the thymus; hypertrophy of the adrenals; passive congestion; fatty infiltration; and at times fatty degeneration in the liver and some atrophy of muscle fibers of the heart. There was more or less passive congestion in the pancreas, spleen, ileum, colon, kidneys, and lungs. In the rats which lacked vitamine A in their diet, there were no special outstanding pathological findings. In marked contrast with the lack of vitamine B the livers showed no fatty changes, the adrenals no hypertrophy, and the thymus no atrophy. The control animals proved to be normal as far as histological examination showed.

RELATION OF VITAMINES AND IODINE TO THE SIZE AND DEVELOPMENT OF THE TADPOLE.

By A. D. EMMETT, FLOYD P. ALLEN, and MARGUERITE STURTEVANT.

(From the Research Laboratory of Parke, Davis and Company, Detroit.)

In the light of our previous work, the temperature conditions, the exact food control, and the question of individual variation were especially borne in mind by keeping the tadpoles in an environment where the temperatures could be maintained within reasonable limits, and by keeping each tadpole in a separate vessel so that there could be no possibility of their eating each other.

The results confirmed the previous findings in regard to the size of the tadpoles; namely, that the presence of vitamines bore a direct relation to size, the water-soluble type B being more of a factor than the fat-soluble A. With respect to the development of the hind legs, the tadpoles having the vitamines in their diet did better than those without them. Vitamine B was apparently more essential than vitamine A. However, for the completion of the metamorphosis, iodine in the inorganic form or in the organic form as in the desiccated thyroid gland was essential. The relative values referred to were determined from time to time by measuring the total body length from shadow photographs, and by binocular observation every 10 to 14 days of the stage of development of the hind legs. Calculating the arithmetric mean for each group on the date observed served as the guide to the rate of advancement.

PENTOSE CONTENT OF SOME TISSUES OF MARINE ANIMALS.

By C. BERKELEY.

(From the Marine Biological Station, Nanaimo, B. C.)

The prevalence of complex pentosans and methylpentosans in marine algæ make it a matter of interest to examine the tissues of animals whose diet they make up for compounds of the same class.

Many observations have been made on the occurrence of pentoses in animal tissues[13] but the only systematic survey of the pentose content of various organs in one animal (the ox) is that by Grund.[14] Although guanylic acid and inosinic acid are the only compounds in which it has been shown with certainty that the pentose exists certain workers have stated that complex pentosans or methylpentosans are present in certain mollusks living on algæ.

An examination of various tissues from a variety of marine animals indicated the presence of a substance soluble in boiling water, which while it yielded furfurol was not precipitated by acetic acid from aqueous solution.

Before undertaking a further investigation of the nature of its combination it was thought important to ascertain the relative amounts of pentose in various marine animals.

For this purpose the tissues were first of all thoroughly extracted with alcohol and the air-dried residue was treated by Grund's modification of the method of Tollens and Kröbe for the determination of pentoses, the result being calculated as xylose, although it is probable that most if not all of the pentose is really present as *d*-ribose.

The following amounts of pentose were obtained: *Squalus* (dogfish) pancreas 2.28, spleen 0.67, kidney 0.65, liver 0.72, testis 0.92, heart 0.38, muscle 0.35 per cent; *Thais lamellosa* (gastropod) liver and gonads 1.15 per cent; *Evasterias troschelii* (starfish) liver 0.77 per cent.

Comparison of the results for the dogfish and for the ox as obtained by Grund show approximate correspondence, the most important difference being that there is relatively more pentose in the organs of the former except in the case of the pancreas, where the amounts are about the same in the two animals.

That the relatively high percentage in the liver (and gonads) of *Thais* may indicate that some is present in a polymerized form, is suggested by Röhmann and others for allied species.

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[13] For a summary of literature see Jones, W., Nucleic acids: Their chemical properties and physiological conduct, New York, 1914, 8, 33, 34.

[14] Grund, G., *Z. physiol. Chem.*, 1902, xxxv, 111.

PENTOSAN- AND METHYLPENTOSAN-SPLITTING ENZYMES OF MACROCYSTIS PYRIFERA.

BY C. BERKELEY.

(From the Marine Biological Station, Nanaimo, B. C.)

By the spontaneous fermentation of the giant kelp (*Macrocystis pyrifera*) in the presence of ground limestone, the kelp almost disappears in 2 to 3 weeks and the solution contains considerable quantities of the calcium salts of mono-basic fatty acids.

No free sugar occurs in the plant. The only carbohydrate constituents present in sufficient quantity to account for the acids produced are pentosan and methylpentosan complexes, the former soluble in dilute alkali and precipitated on acidification, the latter soluble in water and precipitated by alcohol.[15] Breakdown of these complexes to simple sugars must precede acid formation, but no such sugar can be detected when the fermentation takes its normal course.

If freshly cut kelp is extracted with boiling water the extract is neutral, has no reducing properties, and contains methylpentosan, but no pentosan. If the kelp has been cut into small pieces a few hours the extract is acid, reduces Fehling's solution, and contains pentosan as well as methylpentosan.

If kelp is kept for a day or so in strong alcohol and subsequently steeped in warm water containing toluene or other antiseptic, free sugar develops rapidly and both pentose and methylpentose are found in solution. After more prolonged contact with alcohol no breakdown of the polysaccharides occurs on subsequently putting the kelp into water.

By extracting fresh kelp rapidly with frequent changes of alcohol, drying at a low temperature, and grinding, a preparation can be obtained which produces free sugar very rapidly on being added to a solution of either the pentosan or methylpentosan complex. No formation of acid occurs if an antiseptic is present.

From these observations the following conclusions are drawn:

1. Kelp contains enzymes which start to break down its polysaccharide constituents as soon as it is cut.

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[15] Hoagland, D. R., and Lieb, L. L., *J. Biol. Chem.*, 1915, xxiii, 287.

2. The enzymes are inactivated by prolonged contact with alcohol.

3. The production of acids sets in, in the normal course of fermentation, as soon as the sugars are set free and is probably due to the action of bacteria adherent to the kelp.

DIRECT DETERMINATION OF SODIUM IN TISSUES AS SODIUM-CESIUM-BISMUTH-NITRITE.

By R. D. BELL and E. A. DOISY.

(From the Laboratories of Biological Chemistry, Washington University Medical School, St. Louis.)

The insolubility of sodium-cesium-bismuth-nitrite, previously described by Ball,[16] was utilized in the development of a method for the determination of sodium in tissues.

The organic matter is oxidized by a wet ash process with a mixture of nitric and sulfuric acids. Iron must be removed from the mixture of blood salts. The solution is made faintly alkaline to methyl orange and evaporated to 2 or 3 cc. 0.5 cc. of 2 N HNO_3 and an excess of reagent are added. After standing 2 days under illuminating gas, the precipitate is filtered off on a previously weighed Gooch crucible. The reagent is removed by washing with acetone. The crucible is dried at 100° and weighed.

Since the insoluble salt is a nitrite, it may be estimated by titration with potassium permanganate. 1 molecule requires 30 atoms of oxygen for oxidation.

The precipitate contains 3.675 per cent of sodium. 1 mg. of sodium yields 27.2 mg. of the insoluble nitrite.

The reagent is made from cesium nitrate, bismuth nitrate, and potassium nitrite. Any insoluble matter which forms is dissolved by the addition of a little dilute nitric acid.

Good results were obtained in the determination of known amounts of sodium. There was good agreement between results by this method and the indirect perchlorate procedure on blood and urine samples.

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[16] Ball, W. C., *J. Chem. Soc.*, 1910, xcvii, 1408.

UROCHROME EXCRETION AS INFLUENCED BY DIET.

By K. F. PELKAN.

(From the Department of Biochemistry and Pharmacology, University of California, Berkeley.)

Urochrome has long been known as a constituent of urine but its significance has never been definitely determined. Various workers have claimed its relation to the blood and bile colors and to the cellular metabolism of the organism and have denied that it was at all influenced by diet. The amount of experimental evidence submitted in favor of these claims has been small. The present work was undertaken to determine whether and to what extent the amount of urochrome excreted was dependent on the diet. Experiments carried out on a human subject resulted as follows: A low protein diet reduced the urochrome excretion up to 50 per cent, while a high protein diet raised it perceptibly. Gelatin added to a low protein diet did not affect the excretion. Colored substances were found in peptone and in amino-acid digestion mixtures which were similar in all respects to urochrome. All evidence points to the fact that urochrome is to a large extent derived from the proteins of the diet.

RELATION OF EXTRACT OF LUNG TO THE CLOTTING OF BLOOD.

By C. A. MILLS.

(From the Department of Biochemistry, University of Cincinnati, Cincinnati.)

Whereas lung extract, if injected rapidly and in sufficient amounts into animals, causes extensive intravascular coagulation and death usually in less than 1 minute, if injected in small amounts at first and then repeatedly with increasingly larger doses, causes the development of a negative coagulative phase in the blood of the animal. Such blood, withdrawn from the vessels, will not clot spontaneously for days at 5°C., putrefaction usually setting in before clotting occurs. Neither will it clot on treatment with more of the lung extract, calcium salts, thrombin, serum, CO_2 gas, or dilution with water. It differs from peptone plasma in its non-coagulability in that it contains no antithrombin or other material to inhibit the clotting of normal blood or oxalate plasma when added to it, but instead it actually possesses a strong thromboplastic action, probably due to the presence of unchanged lung extract in it.

As much as 1,150 cc. of lung extract have been injected into an 11 kilo dog without causing intravascular clotting, whereas 3 or 4 cc. would kill a dog of this size if injected rapidly in a single dose.

The non-coagulability is not due to the disappearance of fibrinogen from the blood, for the addition of an equal volume of saturated NaCl solution to this plasma always produced a precipitate of fibrinogen. Probably the fibrinogen has been altered by the injections, since it no longer clots with thrombin.

THE RÔLE OF PHOSPHORIC ACID IN CARBOHYDRATE METABOLISM.

By CYRUS H. FISKE.

(From the Biochemical Laboratory, Harvard Medical School, Boston.)

The hourly inorganic phosphate output in man falls and later rises during the morning in the absence of food. After the administration of sucrose while the rate of phosphate excretion is decreasing, the fall is much more marked. The ingestion of sucrose while the rate of phosphate excretion is increasing is likewise followed by a drop in the phosphate output. In either case there is later a compensatory rise, indicating that carbohydrate combustion is accompanied by the retention of phosphate, which is later excreted at an exaggerated rate. It is suggested that this may be an indication that sugar is decomposed in the body *via* a phosphoric acid ester, as in alcoholic fermentation by yeast. The observation may account for the phosphate retention after parathyroidectomy, since the tetany caused by this operation is accompanied by rapid loss of stored carbohydrate.

UREA IN DOGFISH AND SKATE.

By C. C. BENSON.

(From the Biological Board of Control, and the Department of Food Chemistry, University of Toronto, Toronto.)

Determinations of urea in the muscle of dogfish and skate were made on fresh material, on material kept for varying times in cold storage and in the household refrigerator, and on material taken from cold storage and boiled.

In most cases, alcoholic solutions were used for analyses, using methods similar to those of Greene[17] and in others the ammonia was aerated directly.[18]

Fresh dogfish gave 0.5 to 0.6 per cent of nitrogen from urea, specimens from cold storage, 0.4 to 0.57 per cent of urea nitrogen, and boiled specimens showed practically no loss of urea.

Specimens of the muscle of skate, fresh or directly after taking from cold storage, gave 0.6 to 0.8 per cent of urea nitrogen but this value decreased rapidly on keeping in the refrigerator.

The quantities of urea in both forms are too small to be injurious as food, both forms keep well in cold storage, but the skate rapidly forms ammonia from its urea on being thawed.

COMPOSITION OF THE TUBERS, SKINS, AND SPROUTS OF THREE VARIETIES OF POTATOES.

By F. C. COOK.

(From the Bureau of Chemistry, United States Department of Agriculture, Washington.)

Data collected for 20 years by the Vermont, Maine, and New York State Experiment Stations show that an increased yield of potatoes results from spraying potato vines with Bordeaux mixture. This is true whether any *Phytophtora infestans* (late blight of the potato) is prevalent or not.

Because of the high price of copper sulfate in 1915, experiments extending over four seasons were inaugurated by the Department of Agriculture comparing the fungicidal properties of

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[17] Greene, C. W., *J. Biol. Chem.*, 1919, xxxix, 435.

[18] Sumner, J. B., *J. Biol. Chem.*, 1916, xxvii, 95.

Pickering sprays (made with saturated lime water and less copper sulfate than Bordeaux) with standard Bordeaux spray.

Results with Pickering and Bordeaux sprays on two varieties of tubers at Arlington, Virginia, the past season showed an apparent increased yield of tubers and an increased percentage of solids compared with the control. Bordeaux-sprayed potatoes from several states the past season also showed increased yields and increased percentage of solids of the tubers. Green Mountain, Rural New Yorker, and Irish Cobbler tubers stored in the laboratory (average temperature 70°C.) from the time of digging in September, 1918, until sprouting had proceeded to the limit in the Spring of 1919, showed that the sprouts comprised respectively 7.2, 3.5, and 13.3 per cent of the total weight of the tubers, skins, and sprouts. A difference in the concentration or activity of the growth-promoting agencies is suggested. The analytical data for the sprouts, skins, and tubers of these and other samples show little variation in composition for the different varieties of potatoes.

Analyses of sprayed and unsprayed Green Mountain tubers, skins, and sprouts indicate that the spray did not alter the rate of growth or the composition of the sprouts.

The percentage distribution of the nitrogenous substances showed that the sprouts contained more protein, less water-soluble, about the same monoamino and amide nitrogen, and less diamino and other basic nitrogen than the tubers. The sprouts showed a selective action in withdrawing nitrogen, total ash, phosphorus, and water from the tubers in larger proportion than these constituents existed in the tubers.

GLYCOSURIA IN THREE CASES OF CHRONIC NEPHRITIS WITH EDEMA BUT WITH ONLY SLIGHT NITROGEN RETENTION.

By VICTOR C. MYERS and LUDWIG KAST.

(From the Laboratory of Pathological Chemistry and the Department of Medicine, New York Post-Graduate Medical School and Hospital, New York.)

So called "renal diabetes" has been the subject of considerable discussion since Lépine in 1895 postulated the existence of this rather interesting condition, in which the glycosuria is actually the result of renal disease, and not due to hyperglycemia as in diabetes mellitus. About thirty cases have now been recorded in the literature. A few of these cases have shown definite evidence of renal disease aside from the glycosuria, although some would appear to be entirely free from the symptoms ordinarily associated with disease of the kidney. Renal diabetes has been compared with phlorhizin glycosuria, in which condition we have glycosuria without hyperglycemia. Some of the cases, and especially those which we wish to report, find a more direct analogy in the glycosuria of uranium nephritis. Here there is only a mild glycosuria, with a normal or nearly normal glycemia, and a constant proteinuria. Ryffel[19] and Roger[20] have each reported a case similar in some respects to our own. Our three cases (two males. aged 54 and 62, and one female, aged 10) all showed marked proteinuria and edema, but little nitrogen retention, urea nitrogen figures of 11 to 29 mg. per 100 cc. of blood. One of the cases gave a normal blood sugar, but the other two showed a mild hyperglycemia such as is ordinarily encountered in severe nephritis. In one of these the glycosuria was shown by Bailey[21] to be entirely independent of the slight hyperglycemia. The figures for the urine sugar content ranged from negative findings to slightly over 2 per cent, although when sugar was present, it most often amounted to about 0.5 per cent.

A SIMPLIFIED METHOD FOR THE ESTIMATION OF MORPHINE TOGETHER WITH DATA ON THE DISTRIBUTION OF THE ALKALOID IN ACUTE POISONING.

By SERGIUS MORGULIS and VICTOR E. LEVINE.

(From the Biochemical Laboratory, College of Medicine, Creighton University, Omaha.)

Morphine can conveniently be determined in food, tissue, or body fluid by heating with 2 per cent tartaric acid (if solid, the material should first be ground or finely minced) to convert all morphine into the soluble tartarate. The mixture is rapidly cooled, preferably on ice, to solidify the fatty material. The solid residue is removed by straining through cheese-cloth, and washed until the washings are no longer acid to litmus. The liquid, after being filtered through paper, is evaporated to a pasty consistency. The tartarate is then decomposed by the addition of an excess of solid sodium bicarbonate, which sets the alkaloid free. The evaporation is then continued to complete dryness, and the mass powdered and extracted with chloroform to remove the free morphine. The volume of the chloroform extract is noted, and the smallest quantity of the extract is found which on evaporation (in a porcelain crucible over water bath) leaves a residue which yields a definite morphine test. In this way, the relative amount of morphine in several extracts can be determined; knowing the limit of sensitivity of the reaction an approximate estimate of the amount of morphine in the original sample is possible.

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[19] Ryffel, J. H., *Quart. J. Med.*, 1915–16, ix, 91.

[20] Roger, H., *Presse méd.*, 1917, xxv, 337.

[21] Bailey, C. V., *Am. J. Med. Sc.*, 1919, clvii, 221.

The various alkaloidal tests can be applied to the residues after the evaporation of portions of the chloroform extract. The reagent employed—selenium dioxide dissolved in concentrated sulfuric acid—is very sensitive towards the opium alkaloids. It is more sensitive than many of the older reagents.

From experiments on rabbits with acute poisoning the conclusion drawn is that morphine, whether given subcutaneously or by mouth, is widely distributed throughout the animal body, finding its way into almost every tissue. The morphine is invariably found in appreciable quantities in the urine and kidney. Also large quantities may be present in the alimentary tract, liver, lungs, and brain. According to our results especially large amounts of morphine were present in the alimentary tract and excretory organs after administering the poison by mouth, while after injecting under the skin it was recovered principally from the liver, excretory organs, and also from lungs and brain. In view of these findings, it is not feasible to limit the toxicological examination for morphine to the alimentary tract *alone.* An examination of at least the kidney, urine, and liver is also indispensable.

ON THE PROTECTION AGAINST EOSIN HEMOLYSIS AFFORDED BY CERTAIN SUBSTANCES.

By C. L. A. SCHMIDT and G. F. NORMAN.

(From the Department of Biochemistry and Pharmacology, University of California, Berkeley.)

It was found that the inability of gelatin to afford any protection against the photodynamic action of eosin on red cells while marked protection is shown by blood serum, casein, edestin, and certain other proteins can be directly attributed to the lack of tyrosine and tryptophane, since the amino-acids can themselves afford marked protection. The presence of the benzene ring in a molecule does not confer protective ability while the hydroxyphenyl group appears to be one of the determining factors.

EFFECT OF ANESTHETICS ON CELL RESPIRATION.

By J. F. McCLENDON.

(From the Physiological Laboratory, University of Minnesota Medical School, Minneapolis.)

Owing to the difficulties encountered in keeping the metabolism down to the basal level in higher animals, comparative studies in metabolism are desirable. In choosing an animal for such investigations the jellyfish *Cassiopea xamachana* was decided on since the automatic activity of the nervous system may be abolished by cutting off the margin of the bell. The respiration rate is independent of the oxygen tension except at extremely low tensions of oxygen. In determining the rate of respiration, four jellyfish of large size (up to 15 cm. diameter) were deprived of manubrium and bell-margin and placed in a water-tight jar of about a liter capacity and rotated in a thermostat (kept at 30°) for 1 hour. The jar was filled up with sea water of known O_2 and CO_2 content, with the exclusion of air bubbles. The O_2 was determined by the Winkler method and the CO_2 calculated from the alkaline reserve and pH. It was found that the nervous conduction in the bell was anesthetized by 0.5 per cent ether. The jellyfish died at the end of 1 hour in 3 per cent ether, and in less than 1 hour in 4 per cent ether. The respiratory quotient was found to be about 0.95, and since the oxygen could be determined more accurately than the CO_2 only the former will be given. The respiration was measured for 1 hour before anesthetization as a control.

	Oxygen (O_2).							
	0.5 per cent.	1 per cent.			2 per cent.		3 per cent.	4 per cent.
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Ether	2.60	2.40	1.80	2.70	1.80	2.20	1.20	1.20
Control	2.65	2.40	1.80	2.65	2.20	2.10	1.20	2.05

Within the limit of error of the apparatus the O_2 consumption was the same with or without ether, up to 4 per cent in which the jellyfish died before the end of the hour. Carbon dioxide is sometimes considered an anesthetic for marine animals. The following experiments were made by adding CO_2 to sea water of 0.0025 N alkaline reserve and determining the pH at the beginning and end of the experiment and recording the mean value. The pH for the control was always 8.15.

	Oxygen (O_2).				
	pH 6.60.	pH 6.30.	pH 5.80.	pH 5.70.	pH 5.50.
	cc.	cc.	cc.	cc.	cc.
After CO_2	2.00	1.85	1.90	0.70	0.40
Control	1.08	2.05	2.60	1.70	1.70

The addition of CO_2 reduced respiration, but the question arises whether the undissociated CO_2 molecules or the H ions were responsible for this effect. In the following experiments no CO_2 was added to the sea water, but HCl was added thus changing the character of the ionization of the "total CO_2" already present, as indicated by the change in pH. The pH of the control was 8.15.

	Oxygen (O_2).	
	pH 6.60.	pH 5.80.
	cc.	cc.
After HCl	2.02	1.30
Control	2.07	1.85

These experiments show that the CO_2 liberated from the carbonate and bicarbonate by the addition of HCl lowers the metabolism slightly. In order to show the possible effect of change in pH without increasing undissociated CO_2., acid was added to sea water and the CO_2 expelled. The pH was 5.8, and 2.9 cc. of O_2 were used as compared with a control of 2.7 cc. of O_2 in normal sea water of pH = 8.15. Since this is within the limit of error of the apparatus we presume that the H ions were not responsible for the inhibitory effect of CO_2 on respiration of the cells covering the surface of the bell of the jellyfish.

A METHOD OF INCREASING THE SHARPNESS OF THE TONE-MINIMUM IN MEASURING THE ELECTRIC CONDUCTIVITY OF CELLS.

By J. F. McCLENDON.

(*From the Physiological Laboratory, University of Minnesota Medical School, Minneapolis.*)

In measuring the electric conductivity of salt solutions, the sharpness of the tone-minimum is increased by shunting a condenser across the resistance box. Variable condensers sold for that purpose have a maximum capacity of about 0.003 microfarads. Our conductivity cell, when filled with distilled water, has a capacity of less than 0.003 microfarads and a condenser of that capacity may be used with it. When filled with blood, however, such a condenser is entirely inadequate, and owing to the great cost of large variable condensers a home-made apparatus was constructed to increase the sharpness of the tone-minimum. Since this title was sent in, however, we have found that a variable condenser may be built up of telephone condensers at a moderate cost. When our cell was filled with blood a capacity of 1.3 microfarads was required across the box, whereas on the addition of saponin to lake the blood a capacity of 2.2 microfarads was required.

RELATION BETWEEN CHOLESTEROL AND CHOLESTEROL ESTERS IN THE BLOOD DURING THEIR ABSORPTION.

By ARTHUR KNUDSON.

(From the Laboratory of Biological Chemistry, Union University, Medical Department, Albany Medical College, Albany.)

A series of experiments were carried out on dogs, feeding them cholesterol and cholesterol esters, respectively, to determine the relation between the two in the blood during their absorption. In a previous paper, it was shown that, during absorption of a neutral fat (olive oil), there was a marked increase of the cholesterol esters in both the plasma and the corpuscles but no significant change in the total cholesterol.

Cholesterol was determined by the Bloor method, and cholesterol esters by the Bloor and Knudson method. In all the experiments, fat was excluded from the diet. Five experiments were studied in which from 3 to 4 gm. of cholesterol were fed to dogs, and blood specimens taken every 2 hours up to 8 hours after feeding. There was found to be a marked rise in the total cholesterol both in the plasma and in the corpuscles, and in three of the five experiments the increase in the corpuscles was greater. The amount of cholesterol in the combined form as esters remained constant throughout these experiments, indicating that the cholesterol was absorbed in the free form. In a series of four experiments feeding cholesterol esters (such as palmitate, stearate, and oleate) similar increases of the total cholesterol of the plasma and corpuscles were obtained. In three of the four experiments, the increase in total cholesterol was greater in the corpuscles. There was no change in the cholesterol esters in these experiments, indicating that the cholesterol esters must have been hydrolyzed probably in the intestines before being absorbed and that they did not combine again before entering the blood.

THE FORMATION OF ACETONE BODIES FOLLOWING ETHER ANESTHESIA AND THEIR RELATION TO THE PLASMA BICARBONATE.

By JAMES J. SHORT.

(From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York.)

A study of the acetone bodies of the blood and urine was made in twelve cases with the gravimetric methods of Van Slyke and Fitz for blood and Van Slyke for urine. The blood content was but little affected during an average period of 48 minutes of ether anesthesia, but in two cases examined at intervals over a longer period there was a slight increase a few hours later and an increased output in the urine. The CO_2-combining powers in these cases increased even during increase of β-hydroxybutyric acid.

Experimental data indicate that fat in the presence of ether and ether itself may interfere with the accuracy of the method, since both may form a precipitate with the reagents and thus increase the weight of the precipitate formed from the acetone bodies. It could not be shown from analyses on postoperative blood specimens that ether directly effected much error, but through its ability to penetrate the filter it apparently carried with it some fat in solution which caused the increase. Glycerol formed a comparatively large precipitate with the reagents, oleic acid a much smaller precipitate.

It was concluded that, in the cases reported, acetone bodies were not formed promptly enough during ether anesthesia to account for the decreased plasma bicarbonate and that results of analyses for β-hydroxybutyric acid on postoperative bloods may in some instances have been too high, due to an error introduced as a result of the ether content.

PROPERTIES OF HEMOCYANIN AS AN ANTIGEN.

By C. L. A. SCHMIDT.

(From the Department of Biochemistry and Pharmacology, University of California, Berkeley.)

Hemocyanin obtained from the abalone was found to be precipitated by ammonium sulfate within the limits given by Alsberg for that obtained from *Limulus*. On repeated injections into rabbits, positive fixation and precipitin tests were obtained. The substance in moderate doses is non-toxic for guinea pigs.

ENERGY ELIMINATION AND GASEOUS EXCHANGE IN BANANAS, PINEAPPLES, AND APPLES.

By C. F. LANGWORTHY, R. D. MILNER, and H. G. BAROTT.

(From the Office of Home Economics, States Relation Service, United States Department of Agriculture, Washington.)

The changes in ripening fruit are due to enzymes normally present in the fruit and vary with the condition of the surrounding atmosphere in respect to heat, light, water vapor, and other gases. The practical improvement of storage conditions can best be secured by exact knowledge of the biological changes in the materials stored, which in turn implies an increased knowledge of the atmospheric factors just mentioned. The respiration calorimeter in this laboratory is well adapted for the study of such problems and has been used in experiments with apples, bananas, and pineapples. The bananas and pineapples were in the state known in the trade as "active ripening" while the apples were a Fall variety, recently harvested for storage for winter use.

The following table summarizes the results.

Hourly Energy Elimination and Gaseous Exchange per Kilo of Fruit Stored at Room Temperature (10–30°C.).

Fruit.	Energy.	Oxygen.	Carbon dioxide.	Water vapor.	Specific heat.
	cal.	*liters*	*gm.*	*gm.*	
Bananas	0.31	0.06	0.12	0.35	
Pineapples	0.19	0.03	0.07	0.14	
Apples	0.04				0.93

That the changes thus measured are of considerable magnitude appears from a comparison with the energy elimination of the human body at complete rest, which is estimated as 1.1 calories per kilo per hour.

INDICAN IN THE SALIVA OF A CASE OF PELLAGRA COMPLICATED WITH TUBERCULOSIS.

By M. X. SULLIVAN.

(From the Pellagra Hospital, United States Public Health Service, Spartanburg.)

The urine of many of the patients at the hospital was found to contain a high content of indican. Tests were made on the saliva to see if indican could be found therein, using the Obermayer reagent and extracting with chloroform. In Case 313 with large amounts of indican in the urine indican was found likewise in the saliva in two tests. Once the chloroform was blue with indigo blue and once red with indigo red. No other saliva showed the presence of appreciable amounts of indican. It may be noted that shortly after the discovery of indican in his saliva, Case 313 was judged to be suffering from tuberculosis also, from which disease he died about a month later. What part the tuberculosis played in the presence of indican in the saliva cannot be told.

BIOCHEMICAL STUDIES OF THE SALIVA IN PELLAGRA.

By M. X. SULLIVAN and K. K. JONES.

(From the Pellagra Hospital, United States Public Health Service, Spartanburg.)

In pellagra the condition of the mouth, and especially of the tongue, is of considerable importance in establishing a correct diagnosis. The true pellagrous tongue is vividly red and more or less swollen. The literature also speaks of salivation as a symptom of pellagra. In careful quantitative studies at the Pellagra Hospital, Spartanburg, South Carolina, some interesting data were gathered on the saliva in pellagra. It was found that, though there were cases of increased salivary flow, the salivation spoken of by the patients was often apparent rather than real and was seemingly due to some inhibition of swallowing combined with a peculiar ropy change in the saliva or a high content of mucus which made the presence of saliva in the mouth more obvious. Occasionally also the flow was very slow, but in general it was within normal limits which vary considerably. The specific gravity of the saliva of the pellagra patients tended to be higher than that of the controls. The total solids, ash, organic matter, and mucin of the saliva was greater for the pellagrins than for the controls, but bore no relation to the mouth symptoms. The diastatic power of the saliva of pellagrins varied within the limits established by the controls. The sulfocyanate content was much less marked in the saliva of the pellagra patients than in that of normal people. The reaction of the saliva in pellagra was found to be somewhat more alkaline than that of normal saliva.

IS FIBRINOGEN FORMED IN THE LIVER?

By A. P. MATHEWS.

THE ANTIKETOGENIC ACTION OF GLUCOSE.

By P. A. SHAFFER.

A NEW QUALITATIVE AND QUANTITATIVE COLOR REACTION FOR AMINO-ACIDS.

By OTTO FOLIN and H. WU.

ON THE DETERMINATION OF BLOOD SUGAR.

By S. R. BENEDICT and ELIZABETH FRANKE.

A CONVENIENT PERMANENT UREASE PREPARATION.

By OTTO FOLIN.

ON THE LIPINS OF HUMAN CORPUS LUTEUM.

By JACOB ROSENBLOOM.

OBSERVATIONS ON THE CHEMICAL PATHOLOGY OF THE BLOOD IN PERNICIOUS ANEMIA.

By AMOS W. PETERS and A. S. RUBNITZ.

THE EFFECTS OF MODERATE HEMORRHAGE, ETC. ON SOME OF THE BLOOD ELEMENTS AND CONSTITUENTS.

By E. S. SUNDSTROEM and W. R. BLOOR.

ABSTRACT OF THE EXECUTIVE PROCEEDINGS OF THE FOURTEENTH ANNUAL MEETING.

New Members: M. H. Fischer, C. W. Greene, M. J. Hanke, P. J. Hanzlik, A. F. Hess, H. T. Karsner, K. K. Koessler, E. R. Long, W. S. McEllroy, H. H. Mitchell, W. H. Peterson, C. L. A. Schmidt, J. B. Sumner, E. S. Sundstroem, R. W. Williams.

Resignation Accepted: J. T. Halsey.

Incorporation of the Society: Pursuant to a resolution adopted at the previous annual meeting, a special meeting of the Society was held in New York on June 18, 1919, to take proceedings to incorporate the Society as a membership corporation under the Laws of the State of New York, in order that the Society might take control of the Journal of Biological Chemistry, Inc.

The Society was incorporated September 12, 1919.

New By-Laws: It was necessary to put the greater part of the old constitution in the form of By-Laws, which, as adopted, will be found in the Federation Year-Book.

Journal of Biological Chemistry: The Journal was received from the trustees of the Christian A. Herter Memorial Fund and the owners of the Journal of Biological Chemistry, and a resolution was adopted expressing to them the sincere appreciation of the Society for their gift.

Officers for the Year 1920: *President*, Stanley R. Benedict; *Vice-President*, Donald D. Van Slyke; *Secretary*, Victor C. Myers; *Treasurer*, Harold C. Bradley; *Additional Members of the Council*, Otto Folin, Walter Jones, Lafayette B. Mendel. *Nominating Committee*, W. R. Bloor, L. J. Henderson, Andrew Hunter, E. C. Kendall, Graham Lusk, A. P. Mathews, P. A. Shaffer, H. C. Sherman, A. N. Richards.

Dues: The annual dues for the year 1920 were fixed at $2.00.

Forty-four members of the Society attended the meeting.

Victor C. Myers,
Secretary.

PROCEEDINGS

OF THE

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

FIFTEENTH ANNUAL MEETING
Chicago, Ill.
December 28–30, 1920

ABSTRACTS OF PAPERS
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For the List of Members and the Constitution and the By-Laws of the Society see the 1920 Register of the Federation of American Societies for Experimental Biology comprising the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, and the American Society for Experimental Pathology.

Reprinted from The Journal of Biological Chemistry, Vol. XLVI,
pp. iii–li, 1921 (March)

A PHYSICOCHEMICAL METHOD OF CHARACTERIZING PROTEINS. II.

By EDWIN J. COHN.*

(*From the Laboratories of the Harvard Medical School, Boston.*)

At a previous meeting of this Society (1919) Henderson and Cohn described a physicochemical method of characterizing proteins in terms of their amphoteric constants. We pointed out that although the large number of acidic and basic valences involved and the uncertain molecular weights of the proteins rendered it impracticable to evaluate these constants it was possible to represent their strength graphically by plotting the ratio of acid to protein—and of protein to base—as ordinate, and the hydrogen ion concentration as abscissa. If this ratio is expressed as mols of HCl or NaOH added to 1 gm. of anhydrous isoelectric protein the hydrogen ion concentration corresponding to the ordinate *O* becomes the isoelectric point of the protein.

The titration curve of gluten was then presented, with an estimate of the number of acidic and basic valences required in the construction of a curve to approximate the experimental one at all hydrogen ion concentrations. Since then the writer has determined the titration curve of tuberin and of very pure serum globulin, and Loeb has reported the titration curves of pure gelatin and of recrystallized egg albumin. The titration curves of these characteristic proteins reveal important differences.

Each of the proteins has a different isoelectric point. The hydrogen ion concentration corresponding to the isoelectric point determines the ratio of the acid to the basic dissociation constants of the proteins in the equation: $(H^+)^2/Kw = Ka/Kb$, since, at the isoelectric point, we can express the sum of the active valences by one constant, Ka, and of the active basic valences by Kb.

The slope of a tangent drawn to the titration curve at the isoelectric point graphically represents the apparent strength of the multivalent protein as acid and as base, for the greater the slope of the titration curve of equimolal equivalent protein solutions, the greater the amphoteric constants. However, the strength of the amphoteric constants of a protein appear greater, the smaller its molecular weight. The following are the approximate slopes of tangents to the titration curves of different proteins at their isoelectric points.

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* Fellow in Chemistry of the National Research Council.

	(H^+) at isoelectric point.	*Ka/Kb* at isoelectric point.	Approximate slope of titration curve at isoelectric point.
Gluten	About 1×10^{-7}	1	2
Serum globulin	40×10^{-7}	1,600	10
Potato " (tuberin)	500×10^{-7}	250,000	13
Gelatin	200×10^{-7}	40,000	18
Egg albumin	160×10^{-7}	25,600	19

The slope is smallest for proteins which are insoluble in water at their isoelectric points (gluten, glutenin); steeper for proteins which are slightly soluble (serum globulin, tuberin); steepest for egg albumin which is relatively soluble at its isoelectric point.

Since experiments of another kind suggest that glutenin and serum globulin are precipitated as undissociated molecules at their isoelectric point, and since the degree of dissociation of a protein is a function of the strength of its amphoteric constants the differences manifested by the slopes of the tangents to these protein titration curves at their isoelectric points suggest a physicochemical basis for the present classification of simple proteins.

THE SYNTHESIS AND ELIMINATION OF HIPPURIC ACID IN NEPHRITIS: A NEW RENAL FUNCTION TEST.

By F. B. KINGSBURY and W. W. SWANSON.

(From the Biochemical Laboratory, University of Minnesota Medical School, Minneapolis.)

The rate of hippuric acid synthesis and elimination in normal individuals and in patients suffering from various renal disturbances has been studied, the dosage of sodium benzoate being 2.4 gm. or its equivalent of hippuric acid neutralized with sodium bicarbonate.

After ingestion of the above dosage of sodium benzoate the normal individual will eliminate 90 to 100 per cent of it in the form of hippuric acid within 3 hours, in most of the cases between 95 and 100 per cent.

In cases of chronic nephritis the rate of elimination is much slower, but is almost if not quite the same after benzoate ingestion as after hippurate ingestion. More evidence is brought out that the kidney has little to do with the synthesis of hippuric acid. No unconjugated or free benzoic acid is excreted in any case, normal or otherwise. These results are confirmatory of the work of Kingsbury and Bell with nephrectomized dogs.

Our findings confirm those of Lewis and Karr that hippuric acid is excreted more slowly after hippurate ingestion than after the equivalent benzoate ingestion in normal cases.

In nephritis and other renal disturbances the rate of hippuric acid excretion after benzoate ingestion is much slower than with the normal subject. This *rate of elimination* is used as the renal function test, not the *synthetic ability of the kidney* which was the criterion referred to by earlier investigators, since the kidney apparently is not concerned in the actual process of synthesis.

Our results apparently show that the above renal function test varies in the same general direction as the phenolsulfonephthalein test, being low when the latter is low, etc., but seems to be capable of showing kidney conditions to a much finer degree than the phenolsulfonephthalein test.

A CRYSTALLINE URIC ACID COMPOUND IN BEEF BLOOD.

By ALICE ROHDE DAVIS and STANLEY R. BENEDICT.

(*From the Department of Chemistry, Cornell University Medical College, New York.*)

One of us has reported the presence of a non-protein uric acid combination in beef blood. The compound has been isolated from whole blood which has been freed from protein by heat and acetic acid followed by colloidal iron. The uric acid was quantitatively removed with mercury and the compound precipitated from the filtrate by mercury in the presence of sodium acetate. The mercury was removed with hydrogen sulfide and, after concentrating the mercury-free filtrate, the compound crystallized in colorless square plates. The crystals are soluble in hot water and in a concentration of 10 mg. to 1 cc. or greater form on cooling a stiff, water-clear gel which when seeded crystallizes rapidly. The compound is not precipitated by silver-magnesia mixture but on hydrolysis shows the reactions of uric acid and of a pentose. A quantitative estimation of the uric acid in the compound gives the theoretical figure for a combination of uric acid and a pentose in molecular proportions.

Studies are being continued to include ultimate analysis and identification of the carbohydrate radical.

FURTHER OBSERVATIONS ON THE MECHANISM OF THE KETOLYTIC (ANTIKETOGENIC) ACTION OF GLUCOSE.

By P. A. SHAFFER.

(From the Department of Biological Chemistry, Washington University Medical School, St. Louis.)

The following evidence was presented in support of the hypothesis that the effect of glucose in inhibiting the appearance of the acetone bodies is due to a chemical reaction between some unknown derivative or oxidation product of glucose and acetoacetic acid; the product of the reaction being readily susceptible of further oxidation in the body, whereas acetoacetic acid (formed in the main from fatty acid) is, in the absence of its reaction with the glucose derivative, slowly if at all oxidized in the body. For the complete oxidation of fatty acid the simultaneous oxidation of a definite minimum molecular equivalent, probably 1 molecule, of glucose is necessary.

1. Diets, which in different human subjects barely cause the appearance of traces of acetone in breath and of the three acetone bodies in urine, supply 1 molecule of fatty acid to 1 molecule of glucose from all sources (carbohydrate, protein, and glycerol of fat). Such diets are approximately: 10 per cent protein calories, 10 per cent carbohydrate calories, and 80 per cent fat calories, of the total energy requirement of the subject. Diets containing relatively more carbohydrate do not give rise to acetone bodies in the non-diabetic.

These facts enable a rational calculation of low carbohydrate diets which should avoid acetone body production.

2. The respiratory quotient of fasting subjects, or those receiving such diets as will produce traces of acetone bodies, is usually, *when acetone bodies first appear*, 0.74 to 0.76, which values indicate the oxidation at such times of fatty acid and glucose in approximately equimolecular quantities.

3. *In vitro* experiments show that acetoacetic acid in alkaline solution is very slowly oxidized by hydrogen peroxide, while if glucose, fructose, or glycerol is present, the acetoacetic acid rapidly disappears even at room temperature. 1 molecule (or possibly ½ molecule) of the "ketolytic" substance appears to react with, and accomplish the oxidation (or decomposition) of 1 molecule of acetoacetic acid. This phenomenon is believed to be analogous to the "antiketogenic" action of glucose in the body. Butyric acid, hydroxybutyric acid, and acetone do not appear to give similar reactions with glucose; and lactic acid has *in vitro* no ketolytic action.

THE LIPOID BALANCE IN THE BLOOD.

BY W. R. BLOOR.

(*From the Laboratories of Biochemistry and Pharmacology, University of California, Berkeley.*)

In previous communications to the Society it has been pointed out that there is a relationship between fat, lecithin, and cholesterol and also between cholesterol and its esters, in normal blood, and that this relationship is constant within rather narrow limits for the individual and within wider limits for the species. Various factors, such as the absorption of fat, may disturb the balance but in most cases it is soon restored either by elimination of the disturbing constituent or in case that is not immediately possible (as in lipemia) by raising the level of all the lipoid constituents until the equilibrium is established at a higher level. The present communication is a report of a continuation of the study consisting of examination of the lipoid balance under conditions such as fasting, hemorrhage of various kinds, injection of strong salt solutions, etc. In general it appears that if the experimental procedure is severe enough in its effects to produce a marked disturbance, the balance tends to preserve its constancy although at a higher or lower level than normal while if the disturbance is not severe, one or another of the lipoid constituents may change independently. Lecithin and cholesterol especially tend to preserve a constant relationship to each other while fat is much more variable. There appears to be a threshold both in amount and time within which changes may occur in one of the lipoid constituents without disturbing the others. Thus there may be a moderate change of fat alone for a short time without any change in lecithin or cholesterol. A greater change or longer lasting change in fat will result in changes in the lecithin while cholesterol appears to be least readily affected by changes in fat.

THE NATURE OF BLOOD CLOTTING, AS VIEWED FROM THE ACTION OF TISSUE EXTRACTS.

By C. A. MILLS and G. M. GUEST.

(*From the Biochemical Laboratory, University of Cincinnati, Cincinnati.*)

The addition of lung extract, well sedimented in a centrifuge, and calcium to a citrated horse plasma in various proportions gives fibrin yields varying from 1 to 156 per cent greater than that obtained merely by recalcifying such plasma without tissue extract addition. This added material seems to be chemically bound, since inert suspended material, such as starch paste or diluted boiled egg white, is removed in the washing of the fibrin to a very much greater degree.

The phospholipin of the active tissue coagulant is essential to such fibrin increase, since, if it is removed by benzene at room temperature, no such increases take place and the clotting is greatly inhibited.

Regarding 41.6 per cent of the added active material as phospholipin, it was found that thorough phospholipin extraction of the fibrins never yielded as much phospholipin as it was calculated had entered into the fibrin. That the missing phospholipin is fixed in the fibrin is indicated by the presence of much larger amounts of phosphorus in the phospholipin-extracted fibrin than in the normal fibrin.

Since the protein-phospholipin compound of tissue extracts not only accelerates blood clotting, but also seems to enter directly into the composition of the fibrin, and since the phospholipin is essential to such action and is in part bound in the fibrin in a non-extractable form, it is considered that the union of the tissue substance to the blood fibrinogen takes place through the phospholipin molecules. Calcium also being necessary for clotting and being found in the washed fibrin in definite amounts, it is thought that the union of the phospholipin to blood fibrinogen may be through the calcium atom, both the tissue and blood fibrinogen being negatively charged so as to combine with the doubly positive calcium atom.

Thrombin is considered as an end-product of clotting (Wooldridge, Nolf) and not as a factor in bringing about the normal clotting of blood.

Antithrombin, made artificially by removing the phospholipin from the active tissue coagulant, is similar to the antithrombin of peptone plasma in that it is coagulated by heat and may readily be neutralized by cephalin or tissue extract. When neutralized by cephalin, not only does the antithrombic action disappear, but the action becomes strongly thromboplastic. Thus this antithrombin differs from the active tissue coagulant only in its lack of cephalin.

THE ANTISCORBUTIC PROPERTIES OF COMMERCIALLY DRIED ORANGE JUICE.

By J. F. McCLENDON, W. S. BOWERS, and J. P. SEDGWICK.

(*From the Department of Physiology, University of Minnesota Medical School, Minneapolis.*)

(The method of drying is reported under separate title.) The dried juice was fed to guinea pigs on a basal diet of equal parts of alfalfa meal and white flour. The quantities are expressed in cc. of original juice in order to compare them with fresh orange juice. Thirteen guinea pigs of small size were used. Three on the basal diet declined rapidly in weight and died. One fed 0.25 cc. per day lost 5 per cent in weight in 5 weeks and died of scurvy. One fed 0.5 and another 0.75 cc. maintained weight but died of scurvy. All the others gained in weight and the gain was more or less proportional to the quantity of juice. Those getting 1.25 cc. or more juice never developed scurvy. Since this is about as favorable an outcome as with fresh juice, we conclude that the drying did not significantly impair the antiscorbutic properties of the juice. We fed it to infants.

Juice freed from water-soluble B, colloids (pectin), citric acid, and calcium retained antiscorbutic properties.

THE COMMERCIAL DRYING OF ORANGE JUICE RETAINING ITS ANTISCORBUTIC PROPERTIES.

By J. F. McCLENDON and S. M. DICK.

(*From the Department of Physiology, University of Minnesota Medical School, Minneapolis.*)

A unit or dehydrating cell consists of an octagonal chamber 20 feet in diameter and 20 feet high with 12 hot air ports in three tiers. The upper tier admits air at a velocity of 300 feet per minute, the middle at 600 feet, and the lower 150 feet. The centrifugal spray is in the center and just below the level of the upper tier of ports. It consists of a hollow shaft (admitting the juice) and a head made of a series of concave disks serrated at their margins (12 inches in diameter). Between the disks is a series of grooved collars distributing the juice from the hollow shaft to the concave sides of the disks. The shaft and head rotate on ball bearings 5,000 revolutions per minute and transform the juice into a fine spray or fog. The air currents cause eddies and retard the falling of the spray until it is dried. The exhaust ports are at the bottom of the cell and are so large as not to retard the falling of the dried juice in the dead air space. The incoming air is heated to 55–70°, but the evaporation on the surface of the droplets prevents their reaching this temperature before they fall into the dead air space where the temperature is lower. Since the droplet is exposed but 1 minute to the hot air, any temperature effect on the vitamine is minimized. Orange juice is one-eighth solids. If it is evaporated to one-fifth of the original weight the pectin forms a clot from which a syrup oozes. This separation is not hastened by the centrifuge but is by a filter press. If the juice is evaporated to one-sixth or less, no clot separates. The high content in monosaccharides makes it hygroscopic when dried. The taste is practically unaffected and the dried juice, reconstituted with water, makes a pleasant drink. Cane-sugar may be added before drying.

Milk is dried in the same type of cell and the orange preparation may be added to milk powder to restore the antiscorbutic lost in pasteurization. (The milk is first pasteurized since wild yeasts and some bacteria are not killed by drying.) The orange juice imparts a lactic acid flavor to the reconstituted milk in proportion to the amount added, but the milk does not curdle unless enough citric acid (juice) is added to bring it to the isoelectric point of casein.

THE ANTISCORBUTIC PROPERTY OF SOME DESICCATED FRUIT JUICES.

By MAURICE H. GIVENS and ICIE G. MACY.

(*From the Research Laboratories, Western Pennsylvania Hospital, Pittsburgh.*)

If the proper nutrition of man is to be maintained it is necessary that he receive an adequate intake of the antiscorbutic vitamine as well as the other known dietary essentials. The influence of temperature on this vitamine is marked, especially if the heat is excessive. Therefore it is important to determine whether this essential substance is present in foods after *any* heat treatment.

Desiccated foods are now offered for human consumption. Such dried products have been subjected to more or less heat in their preparation. By feeding them daily to guinea pigs on a diet of oats, bran, and hay we have investigated the antiscorbutic potency of the following substances dehydrated by a known commercial process:[1] lemon juice, raw and raw neutralized; grape juice; tomato juice; raspberry juice; grapefruit juice; and orange juice. All these substances except grape juice and raspberry juice contained a significant amount of the antiscorbutic vitamine.

Whether or not dried foods will retain their antiscorbutic potency after long ageing is important. We have reported previously that dried orange juice was a satisfactory antiscorbutic agent.[2] It can now be added that this material is still effective in this respect 2 years after dehydration. All the above substances with the exception of dried raw lemon juice were from 14 to 20 months old. Details of this work as well as a study of dried strawberry and pineapple juices will be published in the near future.

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[1] Our best thanks are due the Merrell-Soule Company, Syracuse, New York, for kindly preparing for us the dried products.

[2] Givens, M. H., and McClugage, H. B., *Am. J. Dis. Child.*, 1919, xviii, 30.

HYDROLYSIS OF LACTALBUMIN.

By CARL O. JOHNS and D. BREESE JONES.

(From the Protein Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.)

Lactalbumin, or the total coagulable protein of milk after removal of the casein, was hydrolyzed by boiling for 4 hours with 20 per cent hydrochloric acid. The following percentages of amino-acids were determined: proline 2.25, aspartic acid 9.37, glutaminic acid 13.00. Evidence has been obtained showing the presence of considerable hydroxyglutaminic acid. One of the most striking results of this hydrolysis is the high yield obtained of aspartic acid, which is over nine times the amount found by Abderhalden and Pribram.[3]

GLYCINE AND GLYCYL-GLYCINE SYNTHESIS.

By J. LUCIEN MORRIS and FLOYD DeEDS.

(From the Biochemistry Laboratory, Western Reserve University School of Medicine, Cleveland.)

The progress of amino-acid formation at successive stages of the reaction of ammonia with monochloroacetic acid was studied. twerntitative determinations of amino nitrogen and total nitrogen Quae made after driving off the ammonia. When aeration was used for the removal of ammonia the subsequent analytical data paralleled Kraut's observations by means of chloride determinations. When ammonia was removed by evaporation with calcium hydroxide, both amino nitrogen and total nitrogen values were irregular. Upon investigation of the irregularities, the conclusion was reached that glycyl-glycine is formed when glycine, monochloroacetic acid, and ammonia are treated with calcium hydroxide at water bath temperature. The formation of glycyl-glycine under these conditions indicates a new method of peptide synthesis which differs from both the diketopiperazine and acid chloride methods. The extent of its application to the experimental preparation of peptides and possible relationships with amino-acid and peptide synthesis in metabolism are being further investigated in this laboratory.

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[3] Abderhalden, E., and Pribram, H., *Z. physiol. Chem.*, 1907, li, 409.

EFFECT OF THE INJECTION OF SMALL AMOUNTS OF ADRENALIN CHLORIDE ON SOME OF THE CHEMICAL CONSTITUENTS OF THE BLOOD.

By ROGER S. HUBBARD and FLOYD R. WRIGHT.

(From the Clifton Springs Sanitarium, Clifton Springs.)

The effect of doses of 0.5 and 1 cc. of a 1 to 1,000 solution of adrenalin chloride on the blood sugar, carbon dioxide-combining capacity of the plasma, and acetone from acetone plus acetoacetic acid, and from β-hydroxybutyric acid in the blood was studied in five normal subjects. It was found that, accompanying the decrease in carbon dioxide-combining capacity of the plasma, there was an increase in both the acetone bodies. This increase lasted for an hour and normal values were reached again after 2 hours, while the lowered carbon dioxide-combining capacity of the plasma persisted for at least 2 hours. No relationship could be demonstrated between increased blood sugar concentration and these changes in acetone and β-hydroxybutyric acid.

THE CONCENTRATION OF AMINO-ACIDS IN THE BLOOD IN RELATION TO FEED AND MILK SECRETION.

By C. A. CARY.

(From the Research Laboratories of the Dairy Division of the United States Department of Agriculture.)

Since it has been shown[4] that the proteins of milk are formed in the mammary gland from the amino-acids of the plasma of the blood one might expect to find some relation between the rate of formation of these proteins and the level of amino-acid N of the plasma. Experiments were tried with milking cows in which the total nutriment and protein of their diet were changed and in which the protein alone or the total nutriment alone was altered.

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[4] Cary, C. A., *J. Biol. Chem.*, 1920, xliii, 477.

As has frequently been found the protein of the diet, the milk yield, the percentage of protein in the milk, and total protein secreted daily varied together directly. In some instances and under some conditions the level of amino-acid N of the plasma varied with these factors. Under other conditions these factors could not be directly correlated with the amino-acid level in either the plasma or the whole blood. This lack of correlation was probably due to variations in the quality of the plasma mixture of amino-acids.

THE ANIMAL UTILIZATION OF PENTOSE.

By ELBERT W. ROCKWOOD and KRIKOR G. KHOROZIAN.

(From the Department of Chemistry, the State University of Iowa, Iowa City.)

Feeding experiments with cats, dogs, rabbits, and man showed that xylose is utilized by carnivorous, herbivorous, and omnivorous animals. The degree of utilization is markedly modified by variations in the rest of the diet, being greatest on a low carbohydrate diet and least when the diet contains much sugar and starch.

THE PHOSPHORIC ACID COMPOUNDS IN THE BLOOD OF NORMAL INFANTS.

By GRACE M. McKELLIPS and ISABEL M. De YOUNG.

(From the Departments of Biochemistry and Pediatrics, University of California, Berkeley.)

The work was undertaken preliminary to the study of these compounds in the blood of infants and young children in certain pathological conditions, especially anemia. The amounts and distribution of the various compounds in the corpuscles were found to be practically the same as in the corpuscles of adults. In the plasma the total phosphate is about 10 per cent lower, inorganic phosphate practically the same, while the lipoid phosphoric acid is 25 per cent lower and the organic considerably higher than in the plasma of adults.

CAN AN INDICATOR METHOD BE USED FOR MEASUREMENT OF CO_2 PRODUCTION FROM ISOLATED NERVE?

By SHIRO TASHIRO and E. M. HENDRICKS.

(*From the Laboratory of Biochemistry, University of Cincinnati, Cincinnati.*)

By immersing nerves in Ringer solution colored with an indicator and by determining extent of the change of the color, it has been claimed by some that the activated nerve does not give off more CO_2 than the resting. Tashiro has demonstrated elsewhere that the activated nerves give off a volatile base—presumably ammonia—which would tend to neutralize the CO_2 formed during excitation. The best way, therefore, to measure CO_2 from isolated nerve and other tissues as well is to estimate it as such and not as acidity produced thereby. If one prefers to use an indicator, three precautions should be taken: (1) Let it respire in CO_2-free air, not in Ringer solution; (2) let it respire in a chamber over non-volatile acid to remove a volatile base formed in the tissue; and (3) unbuffered alkaline solution must be used with the indicator. The new apparatus is presented to show these devices. Although less accurate and less delicate than the original $Ba(OH)_2$ method (biometer), with these devices an indicator method can be used for demonstration that during passage of the nerve impulse, the activated nerve does give off more CO_2 than the resting.

ISOLATION OF CITRIC ACID FROM NORMAL URINE.

By S. AMBERG and MARY E. MAVER.

(*From the Laboratory of the Children's Memorial Hospital, Otho S. A. Sprague Memorial Institute, Chicago.*)

Urine is treated with a slight excess of lead acetate, then ammonia is added to a rather strongly alkaline reaction. The filtrate is evaporated over a small flame to a small volume, whereby it becomes acid. It is treated with H_2S. The excess of H_2S is removed from the filtrate by boiling. On renewed evaporation some crystalline masses separate which are filtered off. To the filtrate Ba acetate is added and just enough $Ba(OH)_2$ to make it partly alkaline. Then about two to three times the volume of 95 per cent alcohol is added. The precipitate is filtered off and washed well. It is extracted with hot water, acidified with H_3PO_4, and the Ba removed with H_2SO_4. The solution is made rather strongly acid with H_2SO_4 and evaporated on the water bath under addition of plaster of Paris to dryness. The dry residue is extracted in the Soxhlet with ether. The ether is evaporated and the residue taken up in water. Lead carbonate is added in slight excess under heating. Later NH_3 is added to strongly alkaline solution and filtered. The filtrate is evaporated and treated with H_2S. After removal of the H_2S from the filtrate, mercuric sulfate is added, the precipitate is filtered off, and the Hg removed by H_2S. The solution is again treated with lead carbonate, etc. To the final solution Ba acetate is added. A precipitate is formed which gives the Sabanin-Laskowski reaction and liberates CO when heated on the water bath with H_2SO_4 in a current of CO_2, whereby the solution does not blacken, but remains light yellow. From some of the Ba precipitate the Ba was removed with H_2SO_4 and the solution heated with Denigès' reagent ($HgSO_4$ in H_2SO_4), whereupon $KMnO_4$ solution was added. The precipitate was decomposed with H_2S and the filtrate extracted with ether and evaporated to dryness. The residue gives a strong purple color on addition of $FeCl_3$. It melts at about 117°C. Pure acetone dicarbonic acid melts at 134°C. The Hg was not determined for several reasons, chiefly because the precipitate obtained according to Denigès from citric acid is said not to be of uniform composition. Residue obtained in the manner described by Denigès for the preparation of acetone dicarbonic acid from citric acid gave a melting point of about 117°C. While Denigès gives the Hg content of the Hg compound obtained with acetone dicarbonic acid prepared according to Pechman, he fails to give the melting point either of the acetone dicarbonic acid prepared according to Pechman or of the one prepared in his way. The ether residue obtained from the substance isolated from urine as well as that obtained from citric acid gave the Legal test and the $FeCl_3$ test.

The barium precipitate yields pentabromoacetone. The barium precipitate was not pure. It did not have the water of crystallization or the proper Ba content after drying at 162°. The amount of CO obtained from this Ba precipitate corresponded to about the amount of pentabromoacetone derived from it.

THE ADAPTATION OF THE PENTABROMOACETONE METHOD TO THE QUANTITATIVE DETERMINATION OF CITRIC ACID IN URINE.

By W. D. McCLURE.

In Collaboration with H. McDonald.

(*From the Laboratory of the Children's Memorial Hospital, Otho S. A. Sprague Memorial Institute, Chicago.*)

Several difficulties arise in the quantitative determination of citric acid by the pentabromoacetone method in urine. Urine acidified with H_2SO_4 and treated with an excess of Br yields a precipitate which might interfere, although the treatment with $KMnO_4$ may minimize the possible error arising from this cause. Urine acidified and treated with animal charcoal gives very little of this Br precipitate, but urine treated this way loses citric acid. The Br precipitate is partly soluble in alcohol, ether, and acetone and the solution, on adding water, becomes very turbid. Such precipitate heated to 100–105°C. for 9 hours loses about 25 per cent of its weight, while pentabromoacetone is all lost. Mixtures of pentabromoacetone and this Br precipitate when heated lose a little more weight than corresponds to the amount of pentabromoacetone present. If, therefore, the precipitate obtained by the pentabromoacetone method from the urine is heated for several hours at 100–105°C., the possible error from the admixture of a Br precipitate other than pentabromoacetone is minimized considerably.

STUDIES ON THE INFLUENCE OF PHENYLCINCHONINIC ACID, SALICYLIC ACID, AND SOME OF THEIR DERIVATIVES ON RENAL EXCRETION.

By VICTOR C. MYERS and JOHN A. KILLIAN.

(*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York.*)

It is now well known that the administration of salicylates and phenylcinchoninic acid (cinchophen) results in an increased excretion of uric acid in the urine and a marked drop in the uric acid content of the blood. An effort has been made to obtain further light on the mechanism of the action of these drugs by experiments on both man and animals. Studies on the effect of these drugs, especially cinchophen and the ethyl ester of its methyl derivative (tolysin), on the composition of the blood have been made in a series of more than forty human cases, data on ten cases being presented.

In four cases the blood urea, as well as the uric acid, was influenced. The action on the uric acid was very pronounced in all four cases, while the urea nitrogen dropped from 25 to 29 mg. per 100 cc. to practically normal figures. It has previously been reported that of the three nitrogenous waste products, uric acid, urea, and creatinine, uric acid is the most difficultly eliminated, with urea standing in somewhat of an intermediate position. One would therefore expect that drugs, which have a general stimulating effect on the kidney, would effect the excretion of uric acid first, urea next, and creatinine last. If the observations reported for urea are correct, it would indicate that the action of these drugs on the kidney is not absolutely *specific* for uric acid, as has generally been considered.

Data were presented on three cases of arthritis, which were clinically benefited by the administration of tolysin, although in this case the influence on the uric acid was slight. In one case which received daily for 10 days 200 grains (four times the ordinary therapeutic dose), there was a tendency to return to the initial blood uric acid level. It appears that the analgesic effect of these drugs is unrelated to their influence on renal excretion.

In one case with comparatively slight renal involvement, tolysin apparently had no influence on the blood uric acid.

In no case did the administration of cinchophen or tolysin cause proteinuria, nitrogen retention, or diminished phenolsulfonephthalein output—thus differing from the data reported by others for salicylates.

Some preliminary studies have been made on the action of other phenylcinchoninic acid derivatives.

STUDIES ON LACTIC ACID.

BY S. W. CLAUSEN.

(*From the Department of Pediatrics, Washington University Medical School, St. Louis.*)

A modified Ryffel method is described for 1 or 2 cc. amounts of blood, determining the α-hydroxy acids as lactic acid. Normal figures in children vary from 18 to 35 mg. per 100 cc. and average

25 mg. In cases of alimentary intoxication in infants, the lactic acid is increased; and the alkali reserve decreased. One severe case showed lactic acid of 180 and a carbon dioxide-combining power of plasma of 21 volumes per cent. This acidosis may yield to measures which supply water to the tissues and restore the blood volume and blood flow to normal. Alkaline therapy unless very guarded may result in tetany. A case of severe cardiac decompensation with lactic acid of 160 and CO_2 of 20, recovered symptomatically after bleeding, and without the use of alkalies, the lactic acid falling to 35 and the CO_2 rising to 50.

OSTEOPHAGIA IN CATTLE.

By H. H. GREEN.

(*From the Veterinary Research Division, Pretoria, South Africa.*)

Investigations, under the direction of Sir Arnold Theiler, on lamziekte,[5] a fatal bovine disease in South Africa, have concentrated attention upon a form of bone eating (osteophagia). Affected cattle search for bone debris and eagerly devour their finds though these may still be putrid. Acute osteophagia is displayed by almost all the cattle in particular regions for 10 months of the year, only abating while the grass is still very young. The abnormal craving is shown to be due to phosphorus deficiency in soil and vegetation, can be produced experimentally upon phosphorus-low rations, and removed by administration of phosphorus compounds or by phosphatic manuring. In itself osteophagia is relatively harmless and has no independent mortality incidence, although it may affect the growth of young stock and the milk yield of cows. Its great economic importance arises from its position in the etiological chain of lamziekte, summarized as follows: (1) A toxin which poisons the cattle, (2) produced by toxicogenic saprophytic anaerobes, (3) in carcass debris, (4) which is only eaten by cattle suffering from osteophagia, (5) due to phosphorus deficiency in soil and vegetation.

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[5] A preliminary report by Theiler, in collaboration with Viljoen, Green, du Toit, and Meier, may be obtained from the Director of Veterinary Research, Box 593, Pretoria, Union of South Africa.

THE DETERMINATION OF IODINE IN THE BLOOD AND TISSUES.

By E. C. KENDALL.

(From the Division of Chemistry, Mayo Foundation, Rochester.)

Thyroxin contains 65 per cent of iodine. The maximum concentration of thyroxin in the blood and tissues may be established by determining the amount of iodine in the blood and tissues. Whether 100 per cent of the iodine present is in the form of thyroxin cannot be known, but the iodine content establishes the maximum content of thyroxin. Recent improvements in the determination of iodine have permitted the study of the iodine content of the blood, tissues, and food. The normal content of iodine in the blood and tissues and in certain pathological conditions will be outlined.

RELATION OF THE NERVOUS SYSTEM TO THE PARATHYROIDS.

By FREDERICK S. HAMMETT.

(From the Wistar Institute of Anatomy and Biology, Philadelphia.)

Two series of observations were made of the effect of thyreoparathyroidectomy and parathyroidectomy on the albino rat. The animals of one series were progeny of rats which had for several generations been subjected to constant contact with men so that their natural defensive instincts of fear and rage had been submerged, the result of which was to increase the stability of their emotional neural activities and consequently their metabolism. The other series was made up of rats from the standard colony in which no such regulation had been established, and in which there was evident a condition of excitability expressed as timidity and characteristic irritability with consequent metabolic instability. The first series is designated as "gentled" the second as "untamed." All rats were originally from the same ancestral group. The removal of the parathyroids or of the complete thyroid apparatus from the gentled rats gave but a 16 per cent mortality. Similar operative procedure on the untamed group gave a mortality of 79 per cent, all rats dying in evident parathyroid tetany. No sex variations were obtained. These results are interpreted as being primarily due to differences in stability of the nervous system with consequent differences in metabolic stability and show the great dependency of emotional and metabolically unstable animals on the secretion of the parathyroid glands.

OBSERVATIONS ON THE OCCURRENCE OF CREATININE AND CREATINE IN BLOOD.

By H. JEANETTE ALLEN and STANLEY R. BENEDICT.

(From the Department of Chemistry, Cornell University Medical College, New York.)

Repeated extractions of the protein residue of blood (whether obtained by picric acid precipitation or by heat coagulation) show a continuous yield of creatinine by the colorimetric reaction. A practically minimum yield corresponding to about 0.3 mg. of creatinine per 100 cc. of blood is finally obtained. From certain considerations we may tentatively conclude that about 0.5 mg. of the creatinine found per 100 cc. of blood (beef, dog, or human) is certainly not creatinine.

In connection with creatine determinations in blood we have found that heating with weak acids (picric, acetic) gives a much higher yield of creatine than when hydrochloric acid is employed. This is contrary to the behavior of creatine in pure solution or when added to blood. Details of the work will be published in the near future.

A NEW REAGENT FOR THE ESTIMATION OF SUGAR IN NORMAL AND DIABETIC URINE.

By JAMES B. SUMNER.

With the assistance of Vi A. Graham.

(From the Department of Physiology and Biochemistry, Medical College, Cornell University, Ithaca.)

A new reagent, 4-6-dinitroguaiacol, is reduced to the intensely colored 4-nitro-6-aminoguaiacol when heated with solutions of glucose in the presence of sodium carbonate. The reaction is quantitative and the amount of glucose can be determined by colorimetric comparison of the unknown solution with a standard.

Since dinitroguaiacol itself has nearly the same quality of color as nitroaminoguaiacol, the coloration due to the excess of dinitroguaiacol is subtracted in calculating the results.

The advantages of dinitroguaiacol as a reagent for sugar in urine are due to the fact that it is reduced by no other of the urinary constituents than the reducing sugars. Uric acid and possibly polyphenols cause some increase in color when glucose is present. These substances are removed by using norite.

Parallel determinations by the new method and by the method of Benedict and Osterberg[6] for the estimation of sugar in normal urine have been made upon samples of diabetic, sugar-free diabetic, nephritic, and normal urine.

SOME OBSERVATIONS ON LOSS OF NITROGEN IN MUSCULAR ATROPHY IN MAN AND ANIMALS.

By AMOS W. PETERS and A. E. GUENTHER.

(*From the College of Medicine. University of Nebraska, Omaha.*)

These experiments were made as a beginning on the determination of the nature of the loss of weight incurred by muscular atrophy consequent on nerve section. They resulted from an endeavor to correlate or explain observations we had made on the chemical pathology of human cases of muscular atrophy.

The continuity of the sciatic nerve in one leg of each rabbit was interrupted by the removal of a section of nerve about 2 cm. long. After 3 weeks the rabbits were killed and examined to determine the success of the nerve section and comparison of the extensor and soleus muscles of the two legs showed a very marked diminution in volume of these two muscles in the leg the sciatic nerve of which had been sectioned. Various physical and chemical comparisons were made of the normal and atrophied muscles of eleven rabbits taken individually and subsequently of a group of four rabbits of which the muscles of the same kind were compounded for analysis. The following is a summary of the *averages* of the results obtained.

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[6] Benedict, S. R., and Osterberg, E., *J. Biol. Chem.*, 1918, xxxiv, 195.

	Eleven rabbits, individual determinations.				
	Average loss of weight. Atrophied.	Average loss of water.		Average total N content.	
		Normal.	Atrophied.	Normal.	Atrophied.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Soleus muscle........	From 23.9 to 45.0.	79.0	79.1	3.08	2.66
Extensor, 1st digit...	" 5.81 " 56.7.	78.2	78.8	3.36	3.07
	Four rabbits, group determination.				
Soleus muscle........		78.5	79.1	3.08	2.66
Extensor, 1st digit...		76.4	79.5	3.00	2.20

The total loss of weight in the atrophied muscle is physiological, *i.e.* occurred in the living animal and is quite variable, though by mere inspection even, the loss in size is very marked. The practical equality of the loss of water on desiccation at about 60–70°C. for both normal and atrophied muscles shows that the atrophy is not dependent on loss of water. The results on the total nitrogen content show an unmistakable loss of nitrogen by the atrophied muscle. Our experiments have not as yet been carried far enough to determine what form of combined nitrogen was lost. The loss of nitrogen observed should be compared with similar results obtained by Greene[7] on the muscle of king salmon during the fast of spawning migration. The rabbit muscles show a marked maintenance of physiological capacity although atrophied, as shown by experimental tests (unpublished).

A 4 HOUR METABOLISM PERIOD.

I. THE ELIMINATION OF URINARY NITROGEN AND CHLORINE IN NORMAL MEN AND WOMEN.

By C. FERDINAND NELSON and MAY KINNEY.

(*From the Laboratory of Biochemistry, University of Kansas, Lawrence.*)

The elimination of total nitrogen, urea, uric acid, creatinine, and sodium chloride in nine healthy individuals was measured over 4 hour periods at irregular intervals varying from 3 weeks to 9 months. On a standard meal consisting of three shredded wheat biscuits, 1 pint of whole milk, 50 gm. of cane-sugar, and two glasses of water (preceded by an ordinary breakfast of fruit, toast, and coffee, with or without an egg) average variations in urinary outputs were obtained as follows:

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[7] Greene, C. W., *J. Biol. Chem.*, 1919, xxxix, 435.

	gm.	*per cent of total nitrogen*
Total nitrogen	0.06	
Urea	0.07	1.6
Ammonia	0.014	0.77
Uric acid	0.007	0.2
Creatinine	0.004	0.2
Sodium chloride	0.73	

The practicability of using the above test meal and 4 hour metabolism period in determining kidney functioning is being investigated.

RATE OF NITROGEN ELIMINATION.

By H. O. POLLOCK and W. S. McELLROY.

(From the Department of Physiological Chemistry, School of Medicine, University of Pittsburgh, Pittsburgh.)

The results of experiments on the rate of nitrogen elimination in dogs following the ingestion of protein fed in the form of lean beef are reported. Immediately after the ingestion of the meat the urine was collected at 2 hour intervals by catheterization and blood drawn from a vein at 1 hour intervals. The total nitrogen and urea nitrogen output in the 2 hour urines and the total non-protein nitrogen and urea nitrogen in the 1 hour blood samples were determined by analysis.

A parallelism was found between the curves for the total nitrogen and urea nitrogen output in the urine and the non-protein nitrogen and urea nitrogen in the blood.

The variations in the curves of nitrogen excretion we attribute to variations in the rate of alimentation and absorption, as indicated by the curves for the non-protein nitrogen and urea nitrogen in the blood.

THE OXIDATION OF CYSTINE IN THE ANIMAL BODY.

By HOWARD B. LEWIS and LUCIE E. ROOT.

(*From the Laboratory of Physiological Chemistry, University of Illinois, Urbana.*)

A study of the conditions which determine the oxidation of the sulfur fraction of the cystine molecule has been made. The amino group of the cystine has been protected from deamination by conjugation with phenylisocyanate to form phenyluraminocystine and the behavior of this compound in the organism of the rabbit studied. The phenyluraminocystine was administered *per os* or subcutaneously, in solution in NaOH or Na_2CO_3. After parenteral administration there was a slight increase in the elimination of SO_4 sulfur but the greater portion of the ingested sulfur was eliminated as unoxidized sulfur. After subcutaneous injection no oxidation of any kind was observed, the sulfur of the molecule being recovered in the urine as unoxidized sulfur. Control experiments on the same animals in which cystine was fed or injected showed an almost quantitative oxidation of the sulfur to sulfates. These experiments indicate that the oxidation of the sulfur fraction of the cystine molecule is probably connected with the process of deamination or with the oxy-acids, products of deamination.

THE NUTRITIVE VALUE OF THE PROTEINS OF THE DRIED YELLOW PEA, PISUM SATIVUM.

By CARL O. JOHNS and A. J. FINKS.

(*From the Protein Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.*)

Nutrition experiments with the dried yellow pea, *Pisum sativum*, show that when cooked, and when the other necessary non-protein dietary ingredients are added, it furnishes adequate protein and water-soluble vitamine for the normal growth of albino rats. Cystine is not required, as in the case of the seeds of the genus *Phaseolus*.

THE PROTEINS OF THE TOMATO SEED, SOLANUM ESCULENTUM.

By CARL O. JOHNS and CHARLES E. F. GERSDORFF.

(From the Protein Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.)

Experimental studies on the proteins of tomato seed have shown that a 4 per cent aqueous solution of sodium chloride extracts the maximum amount of protein; namely, 60 per cent of the total protein (calculated from N × 6.25). Fractional precipitation with ammonium sulfate disclosed the presence of two globulins, both high in sulfur, containing 1.07 and 1.80 per cent respectively. No indication of the presence of albumin or glutelin has been found.

FATE OF PHENYLACETYL DERIVATIVES OF THE AMINO-ACIDS IN THE ANIMAL ORGANISM.

By CARL P. SHERWIN and GEORGE J. SHIPLE.

(From the Chemical Research Laboratory, Fordham University, New York.)

Phenylacetyl ornithine was isolated from the excreta after feeding phenylacetic acid to hens. Phenylacetyl glutamine was isolated from urine after the ingestion of phenylacetic acid by human beings. The following compounds were prepared synthetically:

Phenylacetyl glycocoll (phenaceturic acid).
" alanine.
" glutamic acid.
" asparaginic acid.
" asparagine.
" tyrosine.
" leucine.

The tyrosine, alanine, and leucine compounds which had not previously been prepared were studied. All these phenylacetyl derivatives were fed or injected, using dogs, rabbits, chickens, and human beings as subjects. The glutamine, glycocoll, and ornithine compounds proved non-toxic when introduced into the organisms where they do not occur as detoxication products of phenylacetic acid. Phenylacetyl glutamine undergoes partial hydrolysis into phenylacetyl glutamic acid and partial cleavage into phenaceturic acid when fed to the dog. Phenylacetyl ornithine undergoes destruction when fed to the dog or human being, but apparently not when injected. Phenylacetyl alanine, phenylacetyl leucine, and phenylacetyl tyrosine undergo little or no alteration when fed.

A METHOD FOR THE UTILIZATION OF THE NUTRITIVE SALTS OF SEA WATER.

By J. F. McCLENDON.

(From the Department of Physiology, University of Minnesota Medical School, Minneapolis.)

Little is known of the quantities of the minor inorganic constituents of foods or of our requirements of them. There seems likely to be a shortage of some of them unless we use products from seaweed or sea salts. The evaporation of sea water produces an unstable, hygroscopic, bitter, and laxative mass. On heating the residue to complete dryness, HCl is evolved and the formation of insoluble carbonates and double salts abolishes the bitter and hygroscopic properties. My method is to add 6 gm. of H_3PO_4 per liter to ocean water and evaporate by heat to complete dryness. The resulting mass is non-hygroscopic and of pleasant taste and contains possibly every metallic element. It may be used in cooking or as shaker salt, and insures iodine for the thyroid and fluorine for bones and teeth as well as more common elements. It may be added to common salt to prevent caking of the latter and improve its nutritive qualities.

POWDERED SPINACH AND SEA SALTS IN THE DIET OF ADULTS AND INFANTS.

By J. F. McCLENDON and J. P. SEDGWICK.

(From the Department of Physiology, University of Minnesota Medical School, Minneapolis.)

Spinach is difficult to obtain fresh in winter and yet seems indispensable. Carrots are substituted for spinach in winter as an addition to a milk diet. Milk is deficient in iron and pasteurized milk in vitamines. On the basis of calorific units, spinach has ten times as much iron as is contained in carrots. Spinach has twice as much bone-forming mineral and fat-soluble A as is contained in carrots. By drying and reducing to a powder, the digestibility of spinach is increased and its preparation for consumption facilitated. Powdered spinach mixed with 6 per cent of sea salts will keep indefinitely and may be prepared for an infant by boiling in water 2 minutes or more.

Adults may not like the taste of dried spinach and are especially liable to avoid the pot-liquor that contains about half of some of the valuable constituents (vitamines, salts).

Bread was made of yeast and powdered spinach 1 per cent, soy bean meal 10, sea salts 5, and wheat flour 84 per cent. Rats grew at two-thirds the normal rate on this diet alone.

The mixture was standardized on rats. The addition of 5 per cent of powdered spinach doubled the increase in weight of weaned rats on a whole wheat-casein-salt-mixture diet. These rats weighed 30 gm. As the weight increased the percentage of spinach for normal growth decreased so that 1 per cent of spinach was sufficient for rats of 100 gm. With 2.5 per cent of spinach in above bread and substitution of part white with Graham flour the gain was three-fourths the normal rate and was not affected by substitution of corn for wheat.

	Average gain per wk. *ounces*
10 babies	2.3
With spinach	3.2
" " and 1/12 oz. dried (½ oz. fresh) orange juice	5.7
6 babies	2.2
With spinach	4.4
" " and 1½ oz. fresh orange juice	11.2
8 babies	2.4
With spinach	10.9

INFLUENCE OF PHYSICAL AND CHEMICAL TREATMENT ON THE ADSORPTIVE CAPACITY OF FULLERS' EARTHS.

By G. H. A. CLOWES, A. L. WALTERS, and G. B. WALDEN.

(*From the Biochemical Research Laboratory, Eli Lilly and Company, Indianapolis.*)

Following the experiments reported last year on the adsorptive capacity of fullers' earths for alkaloids and their release in physical and biological systems, experiments have been conducted to determine the influence of chemical and physical treatments on the adsorptive capacity and other properties of Lloyd's reagent. The iron and alumina present in Lloyd's reagent may be removed by treatment with sulfuric acid. The adsorptive capacity for alkaloids appears to be a direct function of the amount of iron and aluminum so contained in the Lloyd's, and to be adversely affected by silica.

Water loss on heating causes a similar effect, the adsorptive curve for alkaloids falling off with loss of water.

The toxicity of Lloyd's reagent when introduced into the peritoneal cavity of rats or guinea pigs appears to follow a similar curve showing a rapid falling off in toxicity with loss of iron and aluminum and also with water, but in the case of iron and aluminum a minimum toxicity point is reached when about 60 per cent of the iron and aluminum has been removed.

THE CHEMICAL NATURE OF THE ACTIVE BLOOD COAGULANT OF TISSUE EXTRACTS.

By C. A. MILLS.

(*From the Biochemical Laboratory, University of Cincinnati, Cincinnati.*)

The active blood coagulant of tissue extracts is not cephalin alone as claimed by Howell, since a milky cephalin emulsion will not quicken the clotting of blood any more than will a saturated lung extract diluted 500 to 1,000 times. Furthermore, such a cephalin emulsion injected intravenously into rabbits in doses up to 5 cc. fails to produce any symptoms, while 0.05 cc. of the saturated lung extract kills the rabbit within a minute by intravascular clotting.

The active coagulant is a protein phospholipin compound, having the solubility characteristics of a globulin, and an isoelectric point at a H^+ concentration of $N \times 10^{-5}$ to 10^{-6}. For a moderately strong extract the addition of acid sufficient to give a calculated acidity of 0.002 N produces complete precipitation of the active substance, the supernatant fluid having the above mentioned H^+ concentration. Properly precipitated and redissolved, the entire coagulative activity of tissue extracts can be recovered through this one substance, while the remaining proteins present in the extract are without action.

This purified compound consists of 41.6 per cent of phospholipin and 58.4 per cent of protein. Accepting the usual molecular weight figures for these substances as cephalin 800 and protein 16,000, a molecule of the active compound would consist of 1 protein molecule to which are attached about 14 phospholipin molecules. Both protein and phospholipin fractions are essential to the activity on coagulation for the following reasons:

1. Addition of acid or alkali sufficient to form *m*-proteins while apparently improving the state of solution of the dissolved material, destroys the coagulative activity almost entirely. This is probably only through change in the condition of the protein fraction.
2. Digestion with a lipase-free trypsin solution very rapidly inactivates the material. Digestion with a strong lipase solution almost trypsin-free also produces complete inactivation but at a much slower rate.
3. Extraction of the phospholipin with fat solvents that leave the protein unaltered in its solubility, gives a protein practically phospholipin-free, which acts to inhibit strongly the normal clotting of blood *in vivo* or *in vitro*. Addition of the phospholipin back to this antithrombic protein restores the positive coagulation activity more than 100 times as much as the activity of the cephalin alone could account for.
4. The addition of an excess of fresh lung phospholipin to a lung extract of any strength will increase its coagulative activity about fourfold. This is an increase many thousand times that to be accounted for by such an amount of cephalin acting alone.

THE HYDROLYSIS OF SUCROSE IN THE STOMACH OF MAN.

By HOWARD B. LEWIS AND ROBERT M. HILL.

(*From the Laboratory of Physiological Chemistry, University of Illinois, Urbana.*)

The hydrolysis of sucrose *in vivo* in the human stomach has been studied by the fractional method. Following ingestion of protein (egg white, cottage cheese washed as free as possible from reducing sugars), sucrose has been administered and samples of the gastric contents were removed at 20 minute intervals. These were analyzed for reducing sugar, and for total carbohydrate present after hydrolysis with HCl. Portions were also incubated for 2 hours and reducing sugar was determined after incubation. The results of the experiments showed a slight hydrolysis of sucrose in the first period, with slightly greater hydrolysis in succeeding periods. The velocity with which the sucrose left the stomach, however, made these effects almost negligible in relation to the total amount of sucrose ingested. Although intestinal regurgitation has occurred in almost every case, no evidence was obtained of the presence of an active intestinal sucrase in the gastric juice. The degree of hydrolysis observed seemed to be due entirely to the acidity of the gastric juice and to be negligible for practical considerations.

A VOLATILE SPERM-STIMULATING SUBSTANCE DERIVED FROM MARINE EGGS.

By G. H. A. CLOWES and E. BACHMAN.

(*From the Biochemical Research Laboratory, Eli Lilly and Company, Indianapolis.*)

The eggs of starfish, sea urchins, and sand-dollars give off a volatile substance which exerts, under extraordinary dilutions, a non-specific stimulating influence on sperm and promotes the fertilization of eggs by sperm so stimulated.

The quantity of this substance produced is directly proportional to the number of eggs employed and its production is maintained for a period of several days from mature sea urchins' eggs. It may be distilled from the extract and the distillate exerts an effect almost equal to that of the original extract. It is not destroyed by heating in a sealed tube in an autoclave for several hours in a neutral or alkaline solution, but is weakened by heating with acid.

It is extraordinarily susceptible to the action of oxidizing agents like iodine, 10 minutes contact with an 0.002 N iodine solution prior to removal of excess iodine with thiosulfate lowering the activity of the solution to one-hundredth of its original strength.

Similar sperm-stimulating and fertilization-promoting effects are exerted by upwards of 50 simple organo substances of the type of propyl, allyl, and cinnamyl alcohol, propylene, etc., which function at a dilution of 1:100,000,000 or more. The stimulating substance derived from the eggs is in all probability a mixture of volatile autolytic products. It probably contains unsaturated groups and has a boiling point not far removed from that of water, but on account of the difficulty in procuring sufficient material at the end of the season, no individual volatile substance has thus far been identified.

THE FAT-SOLUBLE VITAMINE.

By H. STEENBOCK, MARIANA T. SELL, E. M. NELSON, and MARY V. BUELL.

(From the Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.)

In harmony with our observations that yellow maize contains an abundance of the fat-soluble vitamine while white maize contains little or none of it, it has now been found that similar relations obtain with carrots, sweet potatoes, and squash. In peas the vitamine content runs parallel to the yellow pigment content of different varieties. Furthermore in the yellow corn kernel the vitamine is localized in the endosperm which in distinction from the embryo contains most of the pigment.

Carotin of constant melting point through a number of crystallizations was always found to induce growth in rats after growth had been suspended by a lack of the fat-soluble vitamine in the diet. In spite of this it is not meant to infer that the vitamine is necessarily a pigment as is evident from the fact that in butters the vitamine content is not of the same order of magnitude as the pigment content. Furthermore in other materials such as cod liver oil an abundance of the vitamine may occur in the presence of little or no pigment.

So far studies of its distribution do not allow conclusions to be drawn as to its nature but physiologically it is suggestive that in the plant kingdom it has now often been found closely associated with yellow pigments. Chemically it is suggestive that light which destroys these pigments also rapidly destroys the vitamine.

Studies of the stability of the fat-soluble vitamine as extracted from alfalfa hay have indicated that it is not destroyed by saponification in the hot as carried out in the determination of the Koettstorfer number, nor is it destroyed by oxidation with hydrogen peroxide or reduction with nascent hydrogen. Crystalline acetyl derivatives of constituents in the non-saponifiable vitamine fraction have been prepared without resultant destruction of the vitamine which apparently suggests means which can be adopted in the further fractionation of the crude vitamine preparation.

AMINO-ACIDS IN NUTRITION.

III. IS PROLINE A GROWTH-LIMITING FACTOR IN THE PROTEINS OF PEAS?

By BARNETT SURE.

(*From the Department of Agricultural Chemistry, University of Arkansas, Fayetteville.*)

McCollum, Simmonds, and Parsons[8] have recently demonstrated that zein supplements the proteins of peas. Zein is entirely lacking in tryptophane and lysine and probably has very little, if any, cystine, and, since that protein belongs to the class called prolamines, it was thought that it might possess its supplementing value by virtue of its high proline content. It is true, zein is also high in leucine and is quite satisfactory for its tyrosine content. If proline should not be the nucleus of the supplementary character, then either tyrosine or leucine or a combination of these amino-acids might be, if implicit faith is to be placed in chemical methods of analysis. Although McCollum, Simmonds, and Parsons found that gelatin does not supplement peas, gelatin being high in proline, it was thought that since it is so indigestible the fragment containing the proline nucleus may escape digestion and hence fail to bring about response; therefore, in view of that possibility, peas were chosen as a further basis for exploring the capacity of the animal body to synthesize the pyrrolidine nucleus of the protein molecule.[10]

No response was obtained to individual additions of proline, leucine, the leucine fraction, composed of alanine, leucine, and valine, cystine, or tyrosine; or to the addition of proline, in the presence of cystine, leucine, and tyrosine. This eliminates, then, alanine, leucine, valine, cystine, tyrosine, proline, lysine, and tryptophane as limiting amino-acids that may be responsible for supplementing the proteins of peas; and, since zein is very low in the diamino-acids of arginine and histidine, it is suggested, particularly in view of the latest findings of Dakin[11] using his refined methods of protein analysis, that zein may contain one or more amino-acids as yet not isolated that are necessary for growth.

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[8] McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1919, xxxvii, 287.

[9] Totani, G., *Biochem. J.*, 1916, x, 382.

[10] Sure, B., *J. Biol. Chem.*, 1920, xliii, 443, 457.

THE EFFECT OF SUBCUTANEOUS INJECTIONS OF SOLUTIONS OF POTASSIUM CYANIDE ON THE CATALASE CONTENT OF THE BLOOD.

By WILLIAM H. WELKER and J. L. BOLLMAN.

(From the Laboratory of Physiological Chemistry, College of Medicine, University of Illinois, Chicago.)

Cyanide is generally supposed to act by paralyzing the oxidases of the cells. It has been shown experimentally to have a marked effect on the catalase of the blood *in vitro*. If the view concerning the nature of the action of cyanide on the living organism is correct, it should have a marked effect on the catalase content of the blood if the latter enzyme is related to oxidase as has been assumed by some investigations.

This work was undertaken to study this point. Our series of eight experiments shows that cyanide, injected subcutaneously, has little or no effect on the catalase activity of the blood.

THE PROPERTIES OF RENAL LIPASE.

By VICTOR E. LEVINE and SALVER A. GIANELLI.

(From the Biochemical Laboratory, School of Medicine, Creighton University, Omaha.)

The lipolytic activity of the kidney was studied with the rabbit, dog, sheep, pig, and cow, using ethyl acetate, ethyl butyrate, methyl salicylate, olive oil, and castor oil as substrates or zymolytes. Chloroform-water extracts of the cortex, upper medulla, and lower medulla (papillary region) served as the source of enzyme.

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[11] Dakin, H. D., *J. Biol. Chem.*, 1920, xliv, 499.

Renal lipase differs from pancreatic lipase in its behavior towards bile. Even in small concentrations bile inhibits markedly the lipolytic activity of the kidney. That the inhibition is due to the bile salts is shown by the findings with the salts of glycocholic and taurocholic acids, which also depress the lipolytic power of the kidney. Renal lipase behaves like hepatic lipase, for the observations of Loevenhart, confirmed by Quinan, Simmonds, and more recently by Wishart,[12] indicate the existence in the bile of an inhibitor for the latter.

The extent of lipolysis depends upon the order in which enzyme, zymolyte, and inhibitor are mixed. Less zymolyte is hydrolyzed when lipase and bile or bile salts are brought together before the addition of zymolyte than is hydrolyzed when bile or bile salt is added to a mixture of lipase and zymolyte. This behavior argues for a protective action exerted by the zymolyte on the enzyme and is an indirect confirmation of the view that a combation of enzyme with its zymolyte precedes enzyme action and that the inhibitor directly affects the enzyme.

Renal lipase resembles pancreatic lipase in the influence exercised by blood serum, which, according to Pottevin, Rosenheim and Shaw-Mackenzie, and Tsuji,[13] contains a substance that accelerates the activity of steapsin. The serum accelerator is, like the bile accelerator or inhibitor, organic in nature and thermostabile; but, unlike the bile substance, it is non-dialyzable and insoluble in alcohol. The order of mixing enzyme, zymolyte, and accelerator is also very important. No increase in lipolysis or a very slight increase is observed when serum is added to a mixture of enzyme and zymolyte. The acceleration, however, is most striking when the following order is adhered to: enzyme, serum, zymolyte. This fact tends to prove that the accelerator acts directly on the enzyme.

The similarity in the action of serum upon the lipase of kidney and of pancreas leads to the belief that the two enzymes contain the same chemical groupings or nuclei in their molecular structure. The dissimilarity in the action of bile salts, however, indicates a difference in the stereoisomeric configuration of the two lipases or a variation in the side chains or substituents in the major groupings of the enzyme molecule. The results of preliminary experiments differentiate sharply the exolipase of the pancreas from the endolipase of other tissues by the contrasting behavior of the bile salts. Serum, however, seems to accelerate all tissue lipases.

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[12] Loevenhart, A. S., and Pierce, G., *J. Biol. Chem.*, 1906–07, ii, 397. Loevenhart, A. S., *J. Biol. Chem.*, 1906–07, ii, 427. Quinan, C., *J. Med. Research*, 1915, xxxii, 73. Simmonds, J. P., *J. Exp. Med.*, 1918, xxviii, 663. Wishart, G. M., *Biochem. J.*, 1920, xiv, 406.

[13] Pottevin, H., *Compt. rend. Acad.*, 1903, cxxxvi, 767. Rosenheim, O., and Shaw-Mackenzie, J. A., *J. Physiol.*, 1910, xl, p. xii. Tsuji, K., *Biochem. J.*, 1915, ix, 53.

Renal lipase is a reversible enzyme. It not only possesses the power to hydrolyze esters like ethyl acetate, ethyl butyrate, methyl salicylate, and glycerides in olive and castor oils, but it is also capable of synthesizing an ester like ethyl butyrate from ethyl alcohol and butyric acid or a glyceride from glycerol and oleic acid. Fatty degeneration of the kidney may be the result of a reversion of the normal lipolytic process.

ACERIN: THE GLOBULIN OF MAPLE SEED (ACER SACCHARINUM).

By R. J. ANDERSON.

With the Collaboration of W. L. Kulp.

(From the Biochemical Laboratory, New York Agricultural Experiment Station, Geneva.)

The principal protein of the seed of the Silver Maple (*Acer saccharinum*) has been isolated and purified. This protein for which we propose the name acerin is a globulin. It could not be obtained in crystalline form but separated on dialysis in small globular particles. The purified acerin is a nearly white, heavy powder which on combustion leaves no weighable ash. A number of analyses were made on different preparations all of which showed close agreement. The average composition of acerin is as follows: C = 51.44; H = 6.80; S = 0.55; N = 18.34; O = 22.87 per cent.

The nitrogen distribution in acerin is shown in the table below:

	per cent
Amide nitrogen	2.53
Humin "	0.15
Basic "	4.86
Non-basic "	10.63

When analyzed by the Van Slyke method it was found that a considerable percentage of the basic nitrogen was present as lysine.

CREATINURIA.

By E. G. GROSS and H. STEENBOCK.

(From the Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.)

Taking advantage of the technique for the study of creatinuria which had previously been developed in this laboratory when it was demonstrated that creatinuria can always be induced in the pig when given a sufficient amount of protein we have now found it possible to demonstrate that arginine administration also causes an increase in the creatine excretion. Cystine likewise was observed to increase the creatinuria but this increase was apparently due to the resultant acidosis as it was never observed when sodium acetate was administered simultaneously. Under similar conditions the casein or arginine creatinuria was not prevented.

A great variation in the ease with which creatinuria was induced in different animals was observed. That this may have been due, in part at least, to differences in the functioning of the thyroid mechanism, as is well known to occur in pigs, was supported by observations indicating the effect of thyroid administration on creatinuria. Thyroid creatinuria was not prevented by sodium acetate administration.

BRAIN AUTOLYSIS AND MEMORY.

By H. C. BRADLEY.

(From the Laboratory of Physiological Chemistry, University of Wisconsin, Madison.)

Examination of a large number and variety of brains shows that autolysis proceeds in the same way and is subject to the same conditions as in all other tissues thus far examined. Increased acidity leads to increased speed and extent of autolysis. Titration figures are about one-third those obtained on an equal amount of liver or thymus tissue. But the total protein of the brain is only about one-half that of liver or other gland tissues, and the neurokeratin and other non-available structural proteins make up a considerable fraction of this. The amount of protein that can act as substratum in autolysis is therefore very much less than in other glands.

The outstanding feature of normal brain tissue is the permanence of its impressions, memory and habit. This permanence is achieved by its remarkably perfect mechanism for preventing conditions leading to brain autolysis. The large blood supply insures against asphyxial conditions and abnormal hydrogen ion concentration. The respiratory center makes available every resource of the organism to combat an abnormal rise in the hydrogen ion level in the brain. With a normal hydrogen ion level maintained, autolysis cannot go on, and the protein framework is not altered.

THE TITRATION OF ORGANIC ACIDS IN MILK.

By BENJAMIN KRAMER and CARL H. GREENE.

(From the Department of Pediatrics, the Johns Hopkins University, Baltimore.)

The Van Slyke and Palmer method for the determination of the titratable organic acids in urine may be applied to milk if protein, fat, part of the milk sugar, phosphates, and carbonates are removed by means of copper sulfate and a suspension of calcium hydroxide. Determinations were made on cow's milk, human milk, protein milk, and buttermilk. 98 per cent of acetic acid added to cow's milk and 90 per cent of added lactic acid were recovered.

The Method.

10 cc. of milk are placed in a 100 cc. volumetric flask and diluted to 50 cc. with distilled water. 2 to 5 cc. of 20 per cent copper sulfate are then added and 10 cc. of a 10 per cent suspension of calcium hydroxide. The mixture should be shaken at intervals of a few minutes. At the end of 15 minutes the volume is made up to 100 cc. with distilled water and the material mixed and filtered with suction through hardened filter paper on a Buchner funnel.

A 50 cc. aliquot representing the organic acid content of 5 cc. of milk is titrated according to the method of Van Slyke and

Palmer.[14] 0.5 cc. of a 1 per cent solution of phenolphthalein is added to the filtrate which has been transferred to a large test-tube (30 mm. in diameter, 200 mm. in length) and 0.1 N hydrochloric acid added until the pink color just disappears (pH = approximately 8). 5 cc. of 0.02 per cent tropeolin OO are then added and the addition of 0.1 N hydrochloric acid is continued until the color matches that of a standard solution in a test-tube of the same dimensions containing 0.6 cc. of 0.2 N HCl, 5 cc. of tropeolin OO solution, and water to a total volume of 60 cc. Sufficient water should be added to the unknown, when the titration approaches the end-point, to make the volume equal that of the standard solution. A correction must be made for the 0.1 N hydrochloric acid used in the blank titration of distilled water from pH 8.0 to 2.7.

CLINICAL METHODS FOR THE QUANTITATIVE DETERMINATION OF CALCIUM AND MAGNESIUM IN SMALL AMOUNTS OF SERUM OR PLASMA.

By BENJAMIN KRAMER and FREDERICK F. TISDALL.

(From the Department of Pediatrics, the Johns Hopkins University, Baltimore.)

A modified McCrudden technique for the quantitative determination of calcium and magnesium has been applied directly to small amounts of blood serum or plasma. A complete report will appear shortly in the *Johns Hopkins Hospital Bulletin.* The procedure is as follows.

The Calcium Method.

1 or 2 cc. of serum or citrated plasma are measured into an ordinary 15 cc. graduated centrifuge tube containing 2 to 3 cc. of distilled water. The tube should be gently agitated after the addition of each drop of serum. 2 drops of 0.01 per cent phenolsulfonephthalein are added followed by 1 drop of N sulfuric acid. 0.1 cc. of 30 per cent ammonium chloride is then added followed by 1 cc. of approximately N oxalic acid. The sample should be shaken after each addition. 0.5 cc. of a saturated solution of sodium acetate is added and the tube allowed to stand for at

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[14] Van Slyke, D. D., and Palmer, W. W., *J. Biol. Chem.*, 1920, xli, 567.

least 1 hour. The pH of the sample at this point is about 5.4 (dibrom-cresol). The sample is then made up to a definite volume, preferably 6 cc., and centrifuged for at least 20 minutes at high speed. The supernatant fluid is blown off by means of a glass tube, the lower end of which is drawn out to a bore of 1 mm. and curved upward. The lower opening in this tube should be at least 3 or 4 mm. above the precipitate. The precipitate is suspended in the residual liquid by stirring with a glass rod. Enough 2 per cent NH_4OH (2 cc. of concentrated NH_4OH diluted to 100 cc.) is then added to bring the volume to 3 cc., care being taken to wash the rod and the sides of the centrifuge tube free of adherent oxalic acid. The tube is then centrifuged for 10 minutes. This procedure is repeated twice, thus making three washings in all. After the third washing the supernatant fluid is blown off, the tube is shaken to suspend the precipitate, and 2 cc. of approximately N sulfuric acid are added. The tube is then warmed in the boiling water bath for a few minutes and titrated with 0.01 N potassium permanganate until a definite pink color persists for at least 1 minute when viewed under a good light against a white background. The strength of the permanganate solution is determined by titrating against an 0.01 N sodium oxalate (Sörensen).

Calculation.—The number of cc. of 0.01 N potassium permanganate used diminished by 0.02 cc. (the blank) multiplied by 0.2 equals the mg. of calcium in the sample.

The Magnesium Method.

5 cc. of the supernatant fluid from the calcium determination corresponding to 1.66 cc. of serum are measured into a 30 cc. beaker, 1 cc. of 2 per cent $(NH_4)_2HPO_4$ solution is added and then 2 cc. of concentrated ammonia. Next day the sample is filtered through a well packed Gooch crucible, washed ten times with 5 cc. of 1:10 NH_4OH (10 cc. of concentrated ammonia to 100 cc. of water), and then twice with 95 per cent alcohol made alkaline with NH_4OH. The crucible is returned to the beaker and dried for a few minutes at 80°C. in the oven.

10 cc. of 0.01 N HCl are added to the crucible and after a few hours the entire material is transferred to a test-tube, centrifuged,

and 5 cc. of the supernatant fluid are measured into a flat bottomed colorimeter tube graduated for 10 cc., which contains 2 cc. of the 0.3 per cent iron thiocyanate solution. The volume is then made up to 10 cc. with 0.01 N HCl, a rubber stopper inserted, and the fluid mixed. A series of standards is prepared by adding varying amounts of a known NH_4MgPO_4 solution to the thiocyanate solution and bringing the volume up to 10 cc. as in the unknown samples. The color is compared by looking through the entire length of the liquid column against a white background.

Calculation.—The calculation is the same as in the method of Marriott and Howland. Reading (cc. of standard solution) $\times 0.01 \times 2 \times \frac{6}{5} \times 50 =$ mg. Mg in 100 cc. serum, when 2 cc. of serum are used. The standard NH_4MgPO_4 solution should contain 0.01 mg. of magnesium per cc.

STUDIES ON THE DIGESTIBILITY OF PROTEINS IN VITRO.

I. THE EFFECT OF COOKING ON THE DIGESTIBILITY OF PHASEOLIN.

By HENRY C. WATERMAN and CARL O. JOHNS.

(From the Protein Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.)

Preliminary work on a method for the comparison of protein digestibilities is described, the possibility of developing an approximately quantitative method is discussed, and further work outlined.

The experiments were made in approximate imitation of conditions of natural digestion. The samples were digested successively with pepsin in 0.1 N acid and trypsin in N/120 alkali and the extent to which proteolysis had proceeded was ascertained by determining amino nitrogen after the digestion. The experiments indicate that phaseolin is rendered more readily digestible by boiling with distilled water. Cooking for 5 minutes gave a detectible increase in digestibility, while cooking for ¾ hour was apparently sufficient to produce the maximum effect.

The finding of Johns and Finks that cooked phaseolin gave better results in feeding experiments than did the raw is apparently to be explained as due to an increase in digestibility.

THE SULFOCYANATE CONTENT OF THE SALIVA AND URINE IN PELLAGRA.

By M. X. SULLIVAN and PAUL R. DAWSON.

(From the Pellagra Hospital, United States Public Health Service, Spartanburg.)

The sulfocyanate content of the saliva and the urine of patients with active pellagra is, as a rule, less than that of the saliva and the urine of the same patients about to be discharged from the hospital as free from the signs and symptoms characteristic of pellagra.

The increase of the sulfocyanate of the saliva and urine seems to be associated with the betterment of the general condition of the patient, with better assimilation, a higher protein metabolism, and presumably a greater detoxifying power of the system as a whole.

The increase of sulfocyanate of the urine at discharge, over that at entrance, however, is not proportional to the increase of the total nitrogen of the urine.

DIALYSIS INDICATOR METHOD FOR THE DETERMINATION OF THE HYDROGEN ION CONCENTRATION OF MILK.

By BENJAMIN KRAMER and CARL H. GREENE.

(From the Department of Pediatrics, the Johns Hopkins University, Baltimore.)

The hydrogen ion concentration of milk may rapidly and accurately be determined by the dialysis indicator method of Levy, Rowntree, and Marriott. 5 cc. of milk are placed in a collodion sac and dialyzed against an equal volume of 0.9 per cent NaCl solution. Tubes are used of such size that the level of the salt solution is above that of the contents of the sac. Dialysis is allowed to proceed for at least 5 minutes. The collodion sac is then removed and a suitable indicator added to the dialysate. The color developed is compared against a series of standard solutions of known pH. The Clark and Lubs series of indicators were used in conjunction with the standard phosphate solutions of Sörensen and the acid phthalate solutions of Clark and Lubs as well as the Gillespie color standards. Identical results were obtained in each case. The indicator solutions used were brom-phenol blue pH 3.0 to 4.6, methyl red pH 4.4 to 6.0, brom-cresol purple pH 5.2 to 6.8, and phenol red pH 6.2 to 7.0, made up according to the directions of Clark and Lubs.

A series of parallel comparative determinations by the colorimetric and gas-chain methods was made on cow's milk, human milk, protein milk, and buttermilk. The agreement between the two methods was excellent.

The pH of the following samples of milk was determined.

Breast milk, 3 samples	6.8, 6.9, 6.9
Cow's " 6 "	6.7, 6.7, 6.7, 6.7, 6.6, 6.5
Protein " 5 "	5.5, 5.9, 5.0, 5.1, 4.8
Buttermilk, 4 "	5.2, 4.4, 4.7, 4.9

Comparison between the Colorimetric and Electrometric Methods for Determination of pH.

Sample.	Electrometric.	Colorimetric.
	pH	*pH*
Breast milk	6.83	6.8
	6.82	
Cow's "	6.45	6.4
	6.45	
Protein "	4.75	4.8
	4.8	
Buttermilk	4.35	4.4
	4.40	

A STUDY OF A CASE OF RENAL GLYCOSURIA.

By ALFRED P. LOTHROP.

(From the Laboratory of Biological Chemistry, Queens University, Kingston, Ontario.)

The subject was a medical student who discovered the presence of sugar during a routine examination of his urine in the laboratory. The blood sugar concentration was 0.078 per cent and showed a normal response to the ingestion of 100 gm. of glucose without any increase in the amount of glucose in the urine. The quantity of urinary sugar never exceeded 12 gm. in a 24 hour sample and was usually less than that. The sugar promptly disappeared from the urine on a restricted diet and could not be increased above 12 gm. by a mixed diet rich in carbohydrate to which 125 gm. of extra sucrose were added. The results indicate that the case is one of so called "renal glycosuria." A more detailed study of the case is contemplated.

THE CHEMISTRY OF NEOARSPHENAMINE.

By GEORGE W. RAIZISS and M. FALKOV.

(From the Dermatological Research Laboratories, Philadelphia.)

Neoarsphenamine is one of the most important remedies in the treatment of syphilis. Very little is known of its chemical composition. It is supposed to be a condensation product of arsphenamine with 1 or 2 molecules of sodium formaldehyde sulfoxylate. In the opinion of the authors, it is important to ascertain whether one or both amino groups of arsphenamine are combined with sulfoxylate. Neoarsphenamine has been analyzed by the authors for arsenic, nitrogen, and sulfur. The distribution of the sulfur was studied in particular. The results show that neoarsphenamine consists of from 58.5 to 69.5 per cent of arsenical condensation products. The balance is accounted for by uncombined sodium formaldehyde sulfoxylate, sodium chloride, and methyl alcohol of crystallization.

Some samples of neoarsphenamine contain only the mono-substituted product, while others consist of a mixture of both the mono- and di-substituted products.

CONPHASEOLIN: A NEW GLOBULIN FROM THE NAVY BEAN.

By CARL O. JOHNS and H. C. WATERMAN.

(From the Protein Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.)

It has been shown that the navy bean contains besides phaseolin (S, 0.30 per cent) a small amount of another globulin, the sulfur content of which is over 1 per cent.

RAPID DETERMINATION OF PROTEIN IN URINE.

BY H. C. BRADLEY AND WILLIAM ELLIOTT.

(*From the Laboratory of Physiological Chemistry, University of Wisconsin, Madison.*)

The sulfur of proteins appears to be a fairly characteristic quantity, and can be used as the basis for quantitative determinations except where the highest accuracy is required. Protein is digested in boiling NaOH, decomposing very quickly, and the sulfide titrated in acid solution by standard iodine. Figures obtained check well with total nitrogen. The method is particularly applicable to the coagulable protein of nephritic urine, which can be estimated by this method in 15 to 20 minutes.

The possibilities of more rapid, though less accurate, colorimetric reactions for the sulfide are being studied with a view to finding a clinical method of greater accuracy than Esbach's test.

STUDIES ON ORGANIC REGULATION.

I. THE URINARY EXCRETION AND BLOOD PICTURE OF THE HIBERNATING FROG.

BY H. C. VAN DER HEYDE.

(*From the Department of Physiology and Physiological Chemistry, School of Medicine, Morgantown, West Virginia.*)

It is remarkable that of the urinary excretion of the frog, which has been the animal of experimentation *par excellence* for centuries, nothing is known, as far as I could detect. The only material of comparison is given in two studies of Denis on fish urine.

One of the causes of this is the lack of method. The methods of Folin and Denis, of Folin and Wu, of Van Slyke, and of others open a wide field of investigation. Another difficulty is the gathering of urine in sufficient quantities. The method used in this study will be given, along with quantitative results, in the full paper to be published later.

It may be sufficient to say that the urinary figures show great irregularity, though they agree amongst the others of the series. That is, the total nitrogen, urea nitrogen, uric acid, creatinine, etc. figures parallel one another throughout. The influence of temperature seems to be very considerable. Moreover, the humidity of the surroundings is a factor of great importance. The almost complete absence of chlorides and of phosphates in connection with the neutral reaction of the urine is one of the questions raised by this work. The figures of the influence of temperature on excretion are compared with those of Krogh and Snyder in analogous cases. The skin excretion was studied in its relation to urinary excretion.

Regarding the blood, some constituents, such as creatinine and creatine, are too small in amount to be quantitatively determined. Jaffe's test is negative on blood filtrates. Satisfactory determinations of the quantities of total non-protein nitrogen, urea, and of uric acid of the blood were made by the methods of Folin and Wu.

The study is being made as a groundwork for future investigation of the problem of regulation in the process of metamorphosis and for the factors involved in hibernation.

AUTOLYSIS OF ERYTHROCYTES.

By WITHROW MORSE.

(From the Department of Physiology and Physiological Chemistry, School of Medicine, Morgantown, West Virginia.)

The following study aims to meet the objections which have been raised against the conclusions of investigators of the enzyme content of erythrocytes; namely, to preclude clearly the participation of other form constituents, the leucocytes and platelets; to insure that no possibility of inhibition by serum occurs; to use an adequate quantitative method rather than the qualitative scheme used by the most convincing of the papers thus far reported; and finally to control the reaction of medium.

Method.

Potassium oxalate sufficient to make a 20 mg. per cent solution with blood was placed in paraffined 50 cc. centrifuge tubes and blood was received in these tubes immediately from a carotid cannula of an etherized dog. The tubes were centrifuged so that no laking occurred, after which the leucocyte and platelet zones, along with sufficient of the erythrocyte layer to insure that all the other form elements were removed, were decanted and discarded. The original volume was restored by the addition of 0.9 per cent saline solution and the centrifuging was repeated three times, making four in all. The washings at this time were colorless.

The blood was divided into two portions, the first having such an amount of concentrated HCl added (1:50 blood) as leaves a slight free acidity after the adsorption of the acid by the blood protein. Xylene was added to inhibit bacterial development. In the second case, the blood was left intact, nothing being added. After sampling for N non-precipitable by tannic acid the flasks were stoppered with cotton and placed in a thermostat and additional sampling was made at 42 hour and at 9 day intervals. A small amount of filterable nitrogen appears in the initial samples, as shown below. The figures are in milligrams of nitrogen per 100 cc. of whole blood.

Sample 1.	*mg.*	Sample 2.	*mg.*
Initial	39	Initial	47
42 hrs	34	42 hrs	34
9 days	50	9 days	42

The exceeding difficulty in obtaining consistent results owing to the viscous mass one has to deal with in filtering introduces the errors which explain the anomalous fact that less nitrogen appears in the 42 hour sample of Sample 1 and throughout Sample 2. The amounts dealt with here are almost insignificant, for the difference in Sample 1 involves 11 mg. of nitrogen, corresponding to 68.75 mg. per 100 cc. of blood protein digested. At the end of the 9 day period, the flasks are without odor and the viscosity does not seem to have been altered, pointing to no alteration in the colloidal dispersion, as we should expect if digestion of the proteins took place. Comparison of these figures with those obtained by Bradley[15] for slowly autolyzing tissues such as muscle will exhibit the insignificance of the 11 mg. of nitrogen increase in the above experiment, remembering that the tannic acid filtrate involves peptide as well as amino nitrogen.

CONCLUSION.

Autolysis of erythrocytes is not demonstrated.

[15] Bradley, H. C., *J. Biol. Chem.*, 1915, xxii, 113.

THE ENZYMES OF THE ABDOMINAL ADIPOSE TISSUE OF THE COMMON TURKEY, MELEAGRIS GALLOPAVO.

By JOSEPH S. HEPBURN.

(From the Constantine Hering Laboratory, Hahnemann Medical College, Philadelphia.)

Study was made of the abdominal adipose tissue (crude gizzard fat) of six fresh, dry picked, air-chilled, dry packed, well bled, uneviscerated hen turkeys. Catalase, lipase (acting on tributyrin), and esterase (acting on ethyl butyrate) were always found. Simple reductase and oxidase, acting on phenolphthalein, were usually present. Tests for oxidases, which act on α-naphthol and on tricresol, and for protease gave negative results. Aldehyde reductase and peroxidases were found in several of the samples.

DEXTROSE CONTENT OF THE HEN'S EGG.

By JOSEPH S. HEPBURN and E. QUINTARD ST. JOHN.

(From the Constantine Hering Laboratory, Hahnemann Medical College, Philadelphia, and the Philadelphia Clinical Laboratory, Philadelphia.)

The procedure devised by Folin and Wu for blood analysis may be applied to eggs (white, yolk, or whole egg) for the preparation of a clear aqueous solution, and for the colorimetric determination of the dextrose content of that solution. A 0.02 per cent solution of dextrose is used in the colorimetric standard. In individual *fresh hennery eggs*, not over 36 hours old, the following maximum, minimum, and average percentages of dextrose were obtained; each average is based on 6 samples: *Whole egg* 0.36 to 0.49, average 0.45; *white free from yolk* 0.29 to 0.57, average 0.47; *yolk free from white* 0.11 to 0.15, average 0.14; *yolk separated from white in the commercial manner* 0.16 to 0.35, average 0.25. In *eggs preserved in sodium silicate solution* for 9 months, the percentage of dextrose in individual whole eggs (0.50), whites free from yolk (0.47 to 0.54), and commercially separated yolks (0.29 to 0.32) apparently lay within the values found for fresh eggs. *Commercial frozen whites* contained 0.41 to 0.43 per cent of dextrose, average of three samples 0.42 per cent; a *commerical frozen whole egg* contained 0.28 per cent of dextrose; the period of cold storage of these samples was unknown. Dextrose was not present in a sample of *putrid white.*

HEAT ELIMINATION AND GASEOUS EXCHANGE IN CELERY AND EGGS DURING STORAGE.

By C. F. LANGWORTHY and H. G. BAROTT.

(From the Office of Home Economics, States Relations Service, United States Department of Agriculture, Washington.)

Some years ago the respiration calorimeter was used for studies of the energy elimination and gaseous exchanges of ripening fruit[16] but the investigations were interrupted by the pressure of other work during the war. Recently similar experiments have been resumed in cooperation with the Bureau of Markets, this time with special reference to providing data fundamental to the handling and storage of food products. The specific heat and heat elimination of vegetables, fruits, and other commodities are factors which are little understood, but which are of great importance because they affect the optimum conditions and the cost of storage. These factors were determined by use of the respiration calorimeter in the case of freshly dug prime celery from the Department's Experimental Farm boxed (crated) for market. The mean specific heat was determined over two temperature ranges, namely 12–16°, and 17–21°C., and amounted to 0.83 and 0.93 compared to water as 1.00. Heat elimination was determined at three temperatures, namely 12.0°, 16.5°, and 21.0°C., and was found to be respectively 0.19, 0.21, and 0.24 calories per kilo per hour. The celery, which was in good condition, was then placed in cold storage for approximately 1 month, when its heat of elimination was again determined at 13.5–17.5°C. and was found to be respectively 0.20 and 0.33 calories per kilo per hour; its mean specific heat over this average temperature was 1.00 compared to water as 1.00. During a continuation of the experimental period for 8 days longer, the celery deteriorated rapidly and at the end was in an advanced stage of decay. The determination of gaseous exchange was difficult, owing to the large elimination of water. The results obtained in these experiments probably are in error in the second decimal place by an unknown amount because of corrections necessarily made for adhering dirt.

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[16] Langworthy, C. F., Milner, R. D., and Barott, H. G., *J. Biol. Chem.*, 1920, xli, p. xlix.

Another series of experiments was made with hens' eggs, the mean specific heat being determined over two temperature ranges, 12.0–15.0°, and 15.0–19.0°C., and found to be 0.64 and 0.71 as compared with water at 1.00. The heat elimination at three temperatures, 12.0°, 15.0°, and 19.0°C., was found to be 0.01, 0.02, and 0.06 calories per kilo per hour. The data obtained were obviously of an entirely different magnitude from those with celery, the eggs showing a small moisture elimination (due to evaporation) and almost no heat output, while the celery gave out very much moisture and much heat.

The results of these experiments, like the earlier ones with apples, provide definite data regarding matters which must be taken into account in storage; the best results in storage will be possible when such definite information supersedes empirical data, a fact appreciated by the storage industry in this country and elsewhere.

THE QUANTITATIVE RELATION BETWEEN THE VITAMINE CONTENT OF FEED EATEN AND MILK PRODUCED.

By J. S. HUGHES, J. B. FITCH, and H. W. CAVE.

(*From the Kansas State Agricultural College, Manhattan.*)

During the past year two cows have been fed a diet, consisting of pearl hominy, tankage, cottonseed meal, and beet pulp, which by experiment has been shown to be very low in each of the three classes of vitamines. During this time each of these cows has gained in weight, improved in appearance, and has produced an apparently normal calf.

The vitamine content of the milk produced by these cows as well as that produced by cows receiving a feed rich in vitamines has been determined by the usual feeding experiments. The results show no difference in the antiscorbutic property of the milk produced by the cows receiving the two kinds of feeds. The water-soluble and the fat-soluble vitamine content of the milk produced by the cows receiving the feed rich in vitamine was decidedly greater than that of the milk produced by the cows receiving the feed low in vitamines.

Butter made from the milk having a high fat-soluble vitamine content contained much more of this vitamine than the butter made from the milk deficient in this vitamine.

BIOLOGICAL ANALYSIS OF THE GEORGIA VELVET BEAN.

By BARNETT SURE AND J. W. READ.

(From the Department of Agricultural Chemistry of the University of Arkansas, Fayetteville.)

Unlike most seeds so far studied, the Georgia velvet bean was found to be very abundant in the fat-soluble vitamine. It proved to be stable in this seed after autoclaving for 1 hour at 15 pounds pressure. Best response was obtained on low levels of seed intake. The proteins of the seed, recently isolated by Johns and Jones, and chemically found by them to be quite satisfactory for the amino-acid content, biologically were found by us to be very deficient in character. Response to additions of casein was found to be greater at a lower level of velvet bean intake. It is suggested, therefore, that like cottonseed, for growth, and wheat, for reproduction, the Georgia velvet bean is toxic in high concentrations for growth. The seed was also found to be poor in salts and low in the water-soluble concentration. The hulls have no supplementary value in the seed. The leaf was found to be the most nutritious part of the whole plant. It furnishes the necessary salts and is fairly abundant in the water-soluble vitamine. The biological value of the whole plant is being studied at the present time.

IODOMETRIC METHODS FOR THE DETERMINATION OF REDUCING SUGARS IN URINE, BLOOD, MILK, AND OTHER SOLUTIONS.[17]

By A. F. HARTMANN AND P. A. SHAFFER.

ON THE INFLUENCE OF MENSTRUATION ON THE FOOD TOLERANCE IN DIABETES MELLITUS.

By JACOB ROSENBLOOM.

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[17] Shaffer, P. A., and Hartmann, A. F., *J. Biol. Chem.*, 1920–21, xlv, 365.

ABSTRACTS OF THE EXECUTIVE PROCEEDINGS OF THE FIFTEENTH ANNUAL MEETING.

NEW MEMBERS: C. V. Bailey, W. M. Boothby, G. D. Buckner, C. A. Cary, W. M. Clark, J. B. Collip, E. A. Doisy, R. A. Dutcher, H. W. Haggard, B. M. Hendrix, R. S. Hubbard, D. B. Jones, J. F. Lyman, L. S. Palmer, Mary S. Rose, R. L. Stehle.

DECEASED MEMBERS: During the year the following resolution regarding the death of Dr. George Pierce was adopted for the Society by the Council and sent to Mrs. Pierce.

> "The American Society of Biological Chemists wishes to express to Mrs. Pierce its deep sympathy for the mutual loss which has come to her and to the Society in the tragic death of her husband and our colleague, Dr. George Pierce."

Action regarding the death of Dr. Samuel J. Meltzer was taken by a Committee appointed by the Chairman of the Federation.

OFFICERS FOR THE YEAR 1921: *President,* Donald D. Van Slyke; *Vice-President,* Philip A. Shaffer; *Secretary,* Victor C. Myers; *Treasurer,* Harold C. Bradley; *Additional Members of the Council,* Stanley R. Benedict, Otto Folin, Walter Jones. *Nominating Committee,* L. J. Henderson, Andrew Hunter, E. C. Kendall, H. B. Lewis, Graham Lusk, E. V. McCollum, L. B. Mendel, A. N. Richards, H. C. Sherman.

DUES: The annual dues for the year 1921 were fixed at $2.00.

Fifty-four members of the Society attended the meeting.

VICTOR C. MYERS,
Secretary.

PROCEEDINGS

OF THE

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

SIXTEENTH ANNUAL MEETING
New Haven, Conn.
December 28–30, 1921

ABSTRACTS OF PAPERS
ABSTRACT OF EXECUTIVE PROCEEDINGS

Volume V
No. 3

PROCEEDINGS OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS.

SIXTEENTH ANNUAL MEETING.
New Haven, Conn., December 28–30, 1921.

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For the List of Members and the Constitution and the By-Laws of the Society see the 1922 Register of the Federation of American Societies for Experimental Biology, comprising the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, and the American Society for Experimental Pathology.

Reprinted from THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. L,
pp. iii–lvi, 1922 (February)

REASONS FOR BELIEVING THAT RESPIRATORY X IS NOT C_H.

BY YANDELL HENDERSON.

(*From the Laboratory of Applied Physiology, Yale University, New Haven.*)

Low oxygen stimulates respiration. So also does CO_2, but through the C_H of the blood. Following the principle of economy of causes most investigators have held that low oxygen must also alter the C_H of the blood or of the cells of the respiratory center itself. Against this view is the fact that low oxygen induces not acidosis but alkalosis; also the fact that CO_2 stimulates respiration even after vagus section, but low oxygen apparently does not. Under morphine the responsiveness to CO_2 is greatly reduced, while low oxygen is still an effective stimulant.

Respiratory *X* is the hypothetical substance occurring in the blood under low oxygen. It is not an acid. Some evidence suggests that it does not act upon the respiratory center but stimulates the pulmonary vagal endings. It might be a sulfur compound, for H_2S is a powerful respiratory stimulant capable of causing overbreathing followed by fatal apnea. After vagus section sulfide is merely depressant; its stimulant action is, therefore, like that which respiratory *X*, by hypothesis, should have upon the pulmonary endings. Sulfide is rapidly oxidized in the blood. It does not act through C_H. Recent, and as yet, unpublished investigations, indicate that low oxygen must act through a substance of these physiological properties, although apparently respiratory *X* cannot be simply H_2S.

The following experiments were mentioned: On Pike's Peak merely squeezing a hand bulb or even opening and closing the fist sufficiently rapidly to fatigue the forearm caused, in Y. H. and some others, but not in all persons, marked hyperpnea. At sea level a similar experiment can be performed, but only on very sensitive subjects, by sawing wood, using both arms vigorously, but no other muscles. The significant fact is that the hyperpnea that is induced is followed by apnea. Apparently some substance, in addition to CO_2, is produced in the overworked and thus in anoxyemic muscles, which passing into the blood causes overbreathing. It is quite certain that the condition induced in the blood by overbreathing is alkalosis (low ratio of H_2CO_3: $NaHCO_3$), not acidosis. Therefore, the substance is not an acid, but a respiratory stimulant of some other type.

CARBON DIOXIDE AS AN INHIBITANT OF CELL GROWTH.

By G. H. A. CLOWES and HOMER W. SMITH.

(*From the Biochemical Research Laboratory, Eli Lilly and Company, Indianapolis.*)

Comparative experiments carried out on dividing sea urchins' eggs indicate that carbon dioxide exerts a vastly greater inhibitory action than mineral acids, and that the effect of carbonic acid bears no relation to the hydrogen ion concentration. In a single series of experiments carried out under comparable conditions, carbon dioxide exerted an inhibitory effect at pH 8, and completely prevented cell division at pH 6.3, while hydrochloric acid exerted little or no inhibitory effect at pH 5.8 and development was still found at pH 4.6.

A similar difference is observed in studying the inhibitory effect of acids on the maturation of the starfish egg. It is suggested that this specific influence of carbon dioxide is attributable to its greater solubility in organo substances than in the aqueous phase and the experiments indicate that carbonic acid may play an extremely important rôle as a specific regulatory substance in cell growth.

THE ERYTHROPOIETIC ACTION OF GERMANIUM DIOXIDE.

By F. S. HAMMETT and J. E. NOWREY, Jr.

(*From The Wistar Institute of Anatomy and Biology, Philadelphia.*)

The subcutaneous injection of germanium dioxide solutions in adult male or female albino rats produces a marked erythrocythemia. There is no evidence of any toxic action of this compound, even when given in doses up to 180 mg. per kilo of body weight. The erythrocythemia persists for at least 5 weeks after the last dose when large doses are given. It persists for at least

2 weeks after the administration of relatively small doses. There is no accompanying leucemia.

Histological preparations of the liver, spleen, and bone marrow show the following effects have been produced. There is in most cases a dilatation of the hepatic capillaries and a relative engorgement of these with erythrocytes. There is no evidence of the taking on by the liver of its pristine erythropoietic function. The impression is given by the sections cut from the spleens that here too there is an increased number of erythrocytes which is accompanied by a more dense accumulation of cells in the Malpighian corpuscles. This, however, is merely an impression and not of significant magnitude to be validly distinctive. There is neither evidence for an increased red cell destruction by the splenic phagocytes nor evidence that new red cell formation is taking place in this organ. In the bone marrow there is ample evidence that this tissue has been stimulated to the formation of a larger number of nucleated erythrocytes than is taking place in the bone marrow of the control animals. This is verified by counts made of these types of cells.

An examination of the smears made for the determination of the differential counts of the white blood cells showed an entire absence of nucleated red cells. Nevertheless, it was found that the smears from those rats which had received the germanium injections contained more young red cells per unit area than did the smears of the controls. The index of the young red cells was the presence of polychromatic staining erythrocytes.

On the basis of these findings it is concluded that germanium dioxide is an erythropoietic agent of remarkable potency and that the source of the erythrocythemia produced by it is not a liberation of cells from some deposit within the organism, but is a formation of new cells by the bone marrow which has been stimulated to increased activity by the compound used.

IS THERE A SUBSTANCE OTHER THAN FAT-SOLUBLE A ASSOCIATED WITH CERTAIN FATS WHICH PLAYS AN IMPORTANT RÔLE IN BONE DEVELOPMENT?

By E. V. McCOLLUM and NINA SIMMONDS,

(From the Laboratory of the Department of Chemical Hygiene, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.)

and P. G. SHIPLEY and E. A. PARK.

(From the Departments of Pediatrics, the Johns Hopkins University, Baltimore, and School of Medicine, Yale University, New Haven.)

A series of experiments has been conducted which was designed to show the comparative values of butter fat and of cod liver oil in protecting young rats against the injurious effect of a pronounced deprivation of calcium. The basal diets contained about one-fifteenth the optimal amount of calcium and nearly the optimum of phosphorus.

Cod liver oil exerts a very marked protective influence, enabling the animals to grow, be fairly fertile, and appear well nourished for a considerable period under dietary conditions where without it they fail to grow at all and die early. We were unable to demonstrate much difference in the degree of protection afforded by 1 and 3 per cent of the oil.

With the same diets containing butter fat instead of cod liver oil very little protection was afforded even when 3, 10, and 20 per cent of fresh butter fat was supplied.

When in a series of experiments the calcium content of the diet was increased by small increments from 0.045 to 0.245 per cent through the addition of calcium carbonate, the differences between the nutritive effects of cod liver oil and of butter fat tend to disappear and do entirely disappear as the calcium content approaches the optimal, which is about 0.641 per cent.

The results suggest that either (*a*) cod liver oil contains in abundance a dietary essential which is contained but sparingly in butter fat, or (*b*) that at least two organic nutritive principles are associated with certain fats, and that both butter fat and cod liver oil contain each, but in proportions widely different.

THE EFFECT OF DIETS VERY HIGH IN PHOSPHORUS AND VERY LOW IN CALCIUM ON THE DEVELOPMENT OF THE BONES IN YOUNG RATS.

By E. A. PARK and P. G. SHIPLEY,

(From the Departments of Pediatrics, the Johns Hopkins University, Baltimore, and School of Medicine, Yale University, New Haven.)

and E. V. McCOLLUM and NINA SIMMONDS.

(From the Laboratory of the Department of Chemical Hygiene, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.)

Rats fed diets low in calcium but high in phosphorus grow slowly and remain stunted. They seem nervous, reacting in an exaggerated manner to slight stimuli. One or two of them died suddenly when handled. The behavior taken in conjunction with the composition of the diet naturally suggested the possibility of tetany.

The bones were thin. The enlargements of the costochondral junctions and ends of the long bones of the limbs were slight or lacking. Fractures were not found.

Microscopically, the bones showed a fairly constant condition. The cartilage was in most instances calcified. The trabeculæ were thin and exceedingly numerous both at the epiphyseal end of the shaft and in the cortex. The trabeculæ were bordered with not very thick zones of osteoid. A thin fibrous tissue invested the trabeculæ, and when it filled in the spaces between the trabeculæ, it gave rise to pictures corresponding to the "fibrous marrow" so commonly seen in rickets. Scattered around the trabeculæ were many mononuclear cells with basophilic granules, evidently derived from the fixed tissues. They were not found in the marrow but only in the vicinity of the trabeculæ; in our experience they are seen in the rat when fed diets which are low in calcium. Everywhere in the trabeculæ were encountered evidences of bone destruction for the most part carried on by small perforating blood vessels resembling those which destroy the cartilage at the cartilage shaft border. Trabeculæ were found which were very well marked by holes or pits with irregular margins and often containing fragments of bone and bone corpuscles. Other trabeculæ have lost an entire side apparently by process of erosion. Evidently when the diet is deficient in calcium, the organism keeps replenishing the calcium in the circulating blood. The histological changes in the bones mentioned were remarkably constant irrespective of the diets employed. The histological changes in the bones which have been mentioned seem, therefore, to have been dependent on the quantities and relations of the calcium and phosphorus present.

Different diets have different rickets-producing properties apart from their contents of calcium and phosphorus. For the production of rickets there must be growth, not growth in weight, but growth of the skeleton. We believe it probable that a certain amount of the fat-soluble organic factor in the diet favors the development of rickets and is essential for its development; it now seems probable that a certain amount of the fat-soluble factor in the diet is essential for the development of the rickets.

Certain diets low in calcium and having phosphorus in concentrations not far from the optimal, when fed to the rat under the experimental conditions already mentioned, produce rickets. When the phosphorus in the diet is increased to high concentrations, whether by the addition of inorganic or organic phosphorus, the pathological condition in the bones appears to recede from rickets. Diets low in calcium but high in phosphorus seem to be, therefore, less effective in the production of rickets than diets low in calcium in which the phosphorus concentration is not far removed from the optimal.

NEW METHOD FOR THE DETERMINATION OF URIC ACID AND EVIDENCE AS TO ITS DIMORPHISM.

By J. LUCIEN MORRIS and A. GARRARD MACLEOD.

(From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland.)

During critical investigation of colorimetric methods for the determination of uric acid certain conclusions were reached concerning the chemical principles underlying the reactions involved. The zinc precipitation of uric acid possesses many advantages over the silver precipitation. Sodium cyanide, in addition to its usefulness in forming double ions with silver or zinc, multiplies the amount of color obtainable from a given amount of uric acid. By use of this multiplying effect a definite maximum color is attained when an excess of cyanide is present. When such a quantity of cyanide is used presence of sodium carbonate is undesirable. Cyanide gives a color with phosphotungstate in the absence of sodium carbonate. Among other conjugated tungstic acids, arsenotungstic acid was prepared and was found to obviate this difficulty. A new method, based upon these observations, was devised which possesses many advantages over former procedures.

Comparative results were secured from a large number of specimens of human blood by application of the new and the older methods. Some cases showed agreement but others showed marked irregularities. These irregularities were higher values obtained by the new method than by the old. All efforts to lower the new method results obtained in these cases failed. A means was found, however, by which the older method results could be increased so as to correspond with the new method results in those cases in which irregularities occurred, but the same means did not increase the values in the cases in which the two methods agreed. The nature of the modification of the old method used for this purpose suggested that the additional value was due to the presence of a second form of uric acid rather than any other substance. Further evidence to substantiate this conclusion is that uric acid was separated as such from large quantities of human blood in amount corresponding to the new method value.

A PHYSICOCHEMICAL METHOD OF CHARACTERIZING PROTEINS. III.

By EDWIN JOSEPH COHN.*

(From the Laboratories of the Harvard Medical School, Boston.)

In two earlier reports to this society we have pointed out: (1) that curves representing the fractional titration of proteins with acids and bases give a physicochemical method of characterizing proteins, and (2) that a comparison of the titration curves of a number of proteins showed differences in slope at their isoelectric points. This difference in slope was correlated with their solubility. It was shown that the slope of the titration curve of egg albumin (a protein that is soluble in water), was steeper at its isoelectric point than those of the slightly soluble globulins, and that the slope of glutenin (a protein insoluble in water but soluble in dilute acids and alkalies), was still less steep.

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* Fellow in Chemistry of the National Research Council.

The titration of proteins, that are largely precipitated in the neighborhood of their isoelectric points, involves the heterogeneous equilibrium between precipitated and dissolved protein. This is being quantitatively studied in a number of cases by analysis of the concentration of total protein, of protein ions, and of hydrogen ions in the liquid phase.

It has been found:

1. that tuberin, euglobulin, pseudoglobulin, and casein have constant solubilities at their respective isoelectric points. The solubility of these proteins has been found to be independent of the amount of undissolved protein present, and approximately equal to:

	Per liter.
	gm.
Tuberin	0.4
Euglobulin	0.2
Pseudoglobulin	0.1
Casein	0.08

This solubility (*S*) must be considered to be made up of undissociated protein molecules, (HPOH), and of the dissociated protein ions, (PH^+) and (POH^-).

2. Upon the addition of an acid or an alkali to one of these proteins the solubility increases. In the case of casein it has been found that the increase in solubility is, within wide limits, proportional to the NaOH added. From this observation it follows that the amount of protein dissociated as base (HP^+) is negligible, and that the solubility of the undissociated molecules (HPOH), remains constant, as long as the heterogeneous equilibrium with the casein precipitate persists.

3. As long as the solubility of the undissociated protein is constant the mass law equation should assume the form:

$$(H^+) \times (BPOH) = K$$

where (BPOH) designates the soluble compound of protein and base, and K the solubility product constant. This equation has been found to hold for casein in the earliest stages of the reaction:

$$(\text{Casein}) + (\text{NaOH}) = (\text{Na caseinate})$$

where the protein may be considered as acting as though it were a monovalent acid. It varies, however, with variation in the protein concentration in a manner that is still being investigated.

The titration curve for casein has recently been determined by Loeb, and confirmed in this laboratory. This curve appears to have the hyperbolic form (characteristic of the solubility product) only for a short distance near the isoelectric point. Thereafter the curve assumes the S-shape characteristic of homogeneous buffer action. If the curve describing the solubility product is extrapolated, on the basis of the constant calculated in the early stages of the reaction, the amount of combination with base due to this group of valences may be subtracted isohydrically from the titration curve. It will then be found that a second curve is obtained still more characteristically S-shaped. By this graphical method casein may be seen to dissociate as an acid of at least two different strengths.

METABOLISM OF CALCIUM AND PHOSPHORIC ACID ON ISORACHITIC DIETS.

By J. F. McCLENDON.

(*From the Department of Physiology, University of Minnesota, Minneapolis.*)

In young white rats the skeleton contains 96 per cent of the Ca and 82 per cent of the P of the body. The P:Ca ratio is 0.5 in the skeleton and 0.6 in the body. Normal rats of from 22 to 45 gm. on a normal diet retained 10 to 18 mg. of Ca per rat per day and 7 to 9 mg. of P. The same rats at a weight of 76 to 110 gm. retained 20 to 37 mg. of Ca and 11 to 14 mg. of P. This diet contained 790 mg. of Ca and 570 mg. of P. When the P is reduced Sherman has shown that rickets develops. The following diets are divided into isorachitic groups. Group 1 produces definite rickets under the conditions of little exercise and light. Group 2 was doubtful because of disease of the animals. Group 3 causes very slow growth of the bones and Group 4 more rapid growth. Rachitic rats suffer from respiratory insufficiency due to small size of chest due to soft ribs.

Group 1.

Diet.	Flour.	Casein.	Spinach.	NaCl	"Lime."	Yeast.	Wheat germ Ca per 100 gm.	P per 100 gm.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mg.*	*mg.*
57	85	10	1	2	2		931	148
59	93	0	1	2	2	2	894	138
60	90	0	6	2	2		971	125
61	91	2	1	2	2	2	905	132

Group 2. Diet 58 = diet 57 + 100 mg. of cod liver oil per rat per day.

Group 3. Diets 57 to 61 + 0.2 per cent flour substituted by KH_2PO_4.

Diet.	Ca	P
62	962	222
64	900	171
65	970	173
66	923	181

Group 4. Diet 63 = diet 62 + 100 mg. of cod liver oil per rat per day.

Litter 12. Body weight 28 to 58 gm. 1 month old when placed on diets.

Rat No.	Sex.	Diet.	P balance P. D.	Ca balance P. D.
			mg.	*mg.*
I	♀	57	−1	+2 Group 1.
III	♂	59	−1	+1
IV	♀	60	−2	+1
V	♀	61	−1	+4
II	♀	58	−3 (Pneumonia).	+2 Group 2.
VI	♂	62	+2	+2 Group 3.
VIII	♀	64	+1	+5
IX	♂	65	+1	+4
X	♀	66	+1	+4
VII	♂	63	+5	+12 Group 4.

THERMAL EFFECTS ACCOMPANYING ALTERATION OF THE O_2 AND CO_2 CONTENT OF BLOOD.

By HOWARD W. HAGGARD.

(*From the Department of Applied Physiology, Yale University, New Haven.*)

Experiments to be reported demonstrate that the reaction of reduced whole blood with O_2 or CO is practically athermic. The combination of laked blood or hemoglobin solutions with either gas is exothermic. The amount of heat actually liberated is influenced by the state of aggregation of the hemoglobin. Results to be reported suggest the probability that there is an endothermic absorption which neutralizes the liberation of heat by the combination of hemoglobin with O_2 or CO. The shift of the equilibrium $HbO_2 + CO \leftrightarrows HbCO + O_2$ under variation of temperature depends upon the relative heating effects of the combination of the iron of hemoglobin with O_2 and CO. Absorption of CO_2 by the blood is attended with the evolution of heat, and the loss of CO_2 by the absorption of heat.

CHANGES IN THE COMPOSITION OF THE IRISH POTATO TUBER DURING GROWTH WITH PARTICULAR REFERENCE TO THE INFLUENCE OF COPPER SPRAYS.

By F. C. COOK.

(*From the Bureau of Chemistry, United States Department of Agriculture, Washington.*)

Three early varieties of potatoes, Irish Cobbler, Early Rose, and Early Ohio, and one late variety, the Green Mountain, were grown in northern Maine during the 1921 season. Some of the plants were sprayed with copper sprays and others received no copper sprays. Analyses of the tubers made during the growing season showed a gradual increase of solids, starch, and nitrogenous constituents. The principal constituent of the tuber is starch, where the chief increase takes place during tuber growth. The increase of nitrogenous constituents was pronounced in the diamino and other base nitrogen group, although the soluble and the coagulable nitrogen groups increased.

The increase of solids, starch, and nitrogenous constituents was greater in the copper-sprayed than in the non-copper-sprayed tubers. The copper-sprayed tubers gave a higher average percentage of ash than the non-copper-sprayed tubers. A decrease of reducing sugar, sucrose, insoluble nitrogen, and insoluble ash was observed as the tubers developed.

The copper sprays showed their favorable effect at the time the first analyses were made, when the tubers were just large enough for analyses, being about 1 inch in diameter. The three early varieties of tubers showed a higher percentage of sugars (reducing sugars plus sucrose) than the Green Mountain tubers, a late variety, at the time of the first and second analyses. It appears that a correlation may exist between the high sugar content in the early stages of tuber development and the rapid growth which the early varieties make.

The copper sprays not only usually give an increased yield of tubers but yield tubers with higher food value; *i.e.*, with more solids which means more starch as the potato is a starch-producing plant. The nitrogenous constituents of the tuber are also increased by the application of copper sprays. These changes were found in 1921 when no *Phytophthora infestans* was present in northern Maine. The influence of the barium-copper spray on the potato appears to be particularly favorable. Data are presented in detail showing for the first time the changes which take place in tubers during their development and the favorable influence of copper sprays on them.

PEPSIN AND TRYPSIN OF TISSUES.

By H. C. BRADLEY.

(From the Laboratory of Physiological Chemistry, University of Wisconsin, Madison.)

The examination of tissues for pepsin and trypsin, as enzymes of the autolytic complex (Dernby) fails to show evidence of either one. Crucial experiments were carried on in which only one or the other of these enzymes could function as a catalyst of protein hydrolysis and in which they were compared to similar digests to which pepsin or trypsin was added. The results confirm the older findings and definitely disprove the presence of these enzymes.

HYDROGEN ION CONCENTRATION IN AUTOLYSIS.

BY A. KOEHLER, E. SEVERINGHAUS, AND H. C. BRADLEY.

(From the Laboratory of Physiological Chemistry, University of Wisconsin, Madison.)

Measurement of the H ion concentration in autolyzing liver shows that at death an H ion concentration of about pH 7+ exists. Acidity develops for 24 hours; reaching a pH of about 6.4 or 6.5. For 10 days this H ion concentration shows a slight progressive diminution to about 6.7 or 6.8. Thereafter there is a very slow but definite increase of H ion concentration, which may at the end of 20 days reach 6.4 or 6.5 again.

If alkali is added to the digest, sufficient to practically stop autolysis (say pH 9.0) there is a rapid increase of H ion during the first 24 hours (to 7.8 in this case) and thereafter a slow progressive increase, so that in 20 days the reaction may approximate 7.0 very closely. The rapid increase of H ion is not accompanied by autolysis nor by sufficient CO_2 production during the first few days to account for the change in reaction. Oxygen absorption during this period is large, however, suggesting the formation of lactates or similar intermediate acid products which neutralize the alkalinity.

Where acid is added sufficient to produce the optimum autolysis (H ion about 4.5 to 5.5) there is much less striking change of reaction. The increased buffer effect of rapid cleavage to amino-acids does not greatly alter the pH. A digest starting at 5.3 reached 5.7 in 4 days and 6.0 in 20 days, showing a gradual tendency of the reaction to approach that of control.

THE RELATIVE VALUE OF THE SOURCES OF BASE FOR THE FORMATION OF SERUM BICARBONATE.

BY EDWARD A. DOISY AND EMILY P. EATON.

(From the Laboratory of Biological Chemistry, Washington University School of Medicine, St. Louis.)

Defibrinated blood which had been equilibrated with 18, 40, and 75 mm. of CO_2 was centrifuged under oil. Serum chloride and bicarbonate were determined and corrections were applied for the change in volume of the corpuscles. The loss of chloride accounted for 65 to 75 per cent of the increase of bicarbonate.

Separated serum was equilibrated with the same tensions of CO_2 and the bicarbonate determined. Our data indicate that not more than 15 per cent of the total increase of bicarbonate of serum in contact with its corpuscles is due to buffers of the serum. Phosphate determinations on the serum permit us to calculate their value as carriers of carbon dioxide. The serum proteins (including amino- and other organic acids) are probably responsible for most of the base of the serum which may be used to combine with carbon dioxide.

The two sources of base studied do not add up to 100 per cent. We are inclined to ascribe the other 10 per cent to a transfer of other anions (sulfate, phosphate, etc.) from serum to corpuscles.

DETERMINATION OF THE BICARBONATE OF THE BLOOD AND PLASMA.

By DONALD D. VAN SLYKE.

(From the Hospital of The Rockefeller Institute for Medical Research.)

Concerning the acid-base balance more complete information can be obtained by determining both the pH and the bicarbonate of the blood as drawn, than by determining only the CO_2 capacity.[1] The bicarbonate concentration may be determined in two ways:

1. By determining the total CO_2 content, and calculating the proportion of CO_2 present as bicarbonate from the plasma pH. The equation of Hasselbalch[2] may be written in the form

$$[\mathrm{BHCO_3}] = [\text{total } \mathrm{CO_2}]\,\frac{10^{\mathrm{pH} - \mathrm{pK_1}}}{10^{\mathrm{pH} - \mathrm{pK_1}} + 1}$$

which may be used for calculation of the $[BHCO_3]$. The values of pK_1 have been determined by Austin, Cullen, Hastings, McLean, Peters, and Van Slyke to be 6.15 for whole blood and 6.10 for plasma.

2. By adding excess acid to plasma, removing the CO_2, and titrating back to the original pH. This method is a modification of Van Slyke, Stillman, and Cullen.[3] 1 cc. of plasma is acidified in a round flask with 5 cc. of 0.01 N HCl, and the CO_2 is removed by whirling the mixture about the flask. The solution is transferred to a test-tube of 20 mm. diameter, where it is titrated. For the transfer 0.9 per cent NaCl is used, and the NaOH and HCl are also made up in 0.9 per cent NaCl solution, to prevent cloudy globulin precipitation at the end-point. The titration is carried to the color of a control tube in which 1 cc. of plasma has been mixed under oil with 20 cc. of 0.9 per cent NaCl solution containing phenol red. The volume of the titrated solution is also brought to 21 cc. at the end of the titration by adding saline solution.

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[1] Van Slyke, D. D., *J. Biol. Chem.*, 1921, xlviii, 153.

[2] Hasselbalch, K. A., *Biochem. Z.*, 1917, lxxviii, 112.

[3] Van Slyke, D. D., Stillman, E., and Cullen, G. E., *J. Biol. Chem.*, 1919, xxxviii, 167.

This determination may be conveniently combined with the colorimetric pH determination[4] since the plasma solution used for the latter may also be used as control for the end-point of the titration.

THE COLORIMETRIC DETERMINATION OF THE pH OF BLOOD PLASMA.

By GLENN E. CULLEN.

(From the Hospital of The Rockefeller Institute for Medical Research.)

The method is based upon the determination of the total correction which, applied to a colorimetric reading at room temperature in a known dilution of plasma or serum, gives the actual pH at 38°C. This correction factor includes correction for protein effect, the dilution effect, and for the temperature effect.

It is most convenient to dilute the plasma in 0.9 per cent saline solution to about 20 volumes. Dilution up to 15 volumes is accompanied by continual increase in pH, but beyond 20 volumes the change is slight. The temperature change between 20–30° is -0.01 pH per degree increase. The correction factor at 20°, based on about 40 parallel determinations performed colorimetrically and electrometrically on both human and horse blood, is -0.18 pH, with an average deviation of ± 0.02 pH. The calculation of the reaction at 38° from the colorimetric determination is as follows:

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[4] Cullen, G. E., *J. Biol. Chem.*, 1922, l, p. xvii.

$$pH_{38^\circ} = \text{colorimetric pH to} + 0.01\ t^\circ - 0.38$$

The determination is carried out as follows: Blood taken under mineral oil, either oxalated or not, is centrifuged in a stoppered tube. The plasma is removed and always kept under oil. Two 20 cc. portions of neutral 0.9 per cent NaCl are placed in clear glass 20 mm. tubes. 7 drops of a 0.04 per cent phenol red solution are added. The saline solution is then covered with oil. With a 1 cc. pipette, graduated to deliver between two marks, 1 cc. portions of plasma are transferred to the two saline tubes. The mixtures are stirred and the color is compared in a comparator block with that of the Sörensen M/15 phosphate standards containing phenol red. These standards are made up at 0.05 pH intervals and the plasma color is read to 0.01 pH. The determinations are accurate to within the combined possible error of the colorimetric and electrometric determinations; *i.e.*, (0.02 + 0.02) = 0.04 pH.

This method may be applied to other protein-CO_2-containing solutions, such as joint fluids, etc.; but in such cases the protein correction must be redetermined.

A METHOD FOR THE DETERMINATION OF THE TITRATABLE ALKALINITY OF BLOOD.

By ISIDOR GREENWALD.

(From the Harriman Laboratory, Roosevelt Hospital, New York.)

The blood is laked, the proteins are precipitated with picric acid, and the mixture is diluted to ten times the volume of blood taken. An aliquot of the filtrate is titrated with 0.01 N NaOH, using methyl red, and neutral red, phenol red, or cresol red, and, finally, thymolphthalein. The total picric acid is precipitated with nitron, filtered, and weighed. The weight is converted into its equivalent volume of 0.01 N NaOH by multiplying by factors obtained by the titration of known amounts of picric acid. The titration of the free acid in the sample is subtracted from this. The difference represents the alkali contributed by the blood to neutralize the picric acid to the reaction shown by the indicator. The values obtained are unaffected by the presence of small amounts of oxalate, by the exact amount of picric acid used, by the degree of saturation of the blood with oxygen or carbon dioxide and do not change rapidly as the blood is allowed to stand. The error is less than ± 2 per cent if the equivalent of 3 cc. of blood be used and is not more than ± 4 per cent if only the equivalent of 1 cc. be used. Normal blood yields the equivalent of from 40 to 47 cc. of N alkali per liter to methyl red and from 29 to 34 cc. to thymolphthalein.

A METHOD FOR THE INVESTIGATION OF TOTAL BASE EXCRETION.

By CYRUS H. FISKE.

(From the Biochemical Laboratory, Harvard Medical School, Boston.)

A method, sufficiently rapid and calling for small enough amounts of urine to meet the requirements of short period metabolism experiments, has been devised for the determination of the sum of all the non-volatile strong bases in urine (sodium, potassium, calcium, and magnesium). It is based partly on well known and long used devices for the separation of these bases from other constituents (ashing with sulfuric and nitric acids, removal of phosphate with ferric chloride, and of the excess iron as the basic acetate) with certain refinements necessitated by the small scale of the method. The bases are eventually obtained as sulfates, and the determination is made by a sulfate estimation with the benzidine method.

PHYTIN AS A SOURCE OF PHOSPHORUS IN THE PREVENTION OF RICKETS.

By WALTER H. EDDY, H. R. MULLER, and HATTIE L. HEFT.

(From the Department of Physiological Chemistry, Teachers College, Columbia University, and the Department of Pathology, New York Hospital, New York.)

The announcement by Sherman and Pappenheimer of two diets (84 and 85) which are respectively rachitic and antirachitic has suggested the desirability of determining whether the antirachitic effect obtained by the addition of 75 mg. of phosphorus in 100 gm. of diet in the form of K_2HPO_4 is paralleled by other forms of dietary phosphorus. Phosphorus occurs in foodstuffs as inorganic phosphorus, as phosphoprotein, as phospholipin, and as phytin. In papers soon to be published Pappenheimer, Zucker, McCann, and Gutman will report findings in regard to phosphoprotein and lecithin. The present paper deals with preliminary work on the rôle of *phytin* in this connection.

Microscopic sections are shown obtained from studies of a single litter of rats which indicate that when an amount of phytin is fed that furnishes about 75 mg. of phosphorus in 100 gm. of the basal diet, *i.e.*, Diet 85 with phytin substituted in place of K_2HPO_4, protection fails. Growth facts and food intake figures are supplied that show that there was little difference in the growth rate of the three series studied and that the food intakes during the 30 day period were practically identical, 9+ gm. daily.

Preliminary results are reported on a much larger series of rats which at the time of preparation of this paper had been distributed over five series of diets as follows: Group I on Diet 84; Group II on Diet 85; Groups III, IV, and V on a diet which was modified by replacing the inorganic phosphate of Diet 85 by varying amounts of phytin, so proportioned as to provide 70, 105, and 140 mg. of phosphorus to each 100 gm. of the diet, respectively.

Figures were given for the inorganic blood phosphorus, as determined by the Bell and Doisy method, after 14 days on this diet. These figures seem to support the conclusions of Hess and Gutman in regard to the diagnostic value of this test as an indicator of rachitic conditions, if we consider solely the two control series. In the case of the phytin-fed rats, however, the figures obtained were extremely variable and failed to support the x-ray evidence. The x-ray evidence while confirming the efficiency and inefficiency of Diets 85 and 84 respectively, shows absence of protection in any of the phytin series. These results seem to suggest that even though we give phytin in amounts sufficient to double the amount of phosphorus supplied by the inorganic phosphate of control Diet 85 it fails to protect.

Previous experience has shown that final judgment should be reserved until microscopic sections are obtained and the above findings are reported as subject to revision in the light of such evidence at the end of the normal period.

THE INFLUENCE OF DIET UPON THE CONCENTRATION OF PHOSPHORUS AND CALCIUM IN THE SERUM OF RATS.

By BENJAMIN KRAMER and JOHN HOWLAND.

(From the Department of Pediatrics, the Johns Hopkins University, Baltimore.)

A study of the calcium and the inorganic phosphorus in the blood serum of rats has been made to determine how the concentration of these substances is affected by diets, especially those producing rickets. We have previously shown that in human rickets the concentration of inorganic phosphorus is low and that it increases during spontaneous cure or as the result of the administration of cod liver oil. The concentration of calcium is essentially unchanged. In rats upon a diet deficient in phosphorus and in the organic factor contained in fats, especially cod liver oil (a diet which regularly produces rickets in the rat) the concentration of inorganic phosphorus is much diminished. The calcium is unchanged as long as a sufficient quantity of this is contained in the diet. When the diet is deficient both in calcium and in the organic factor, the calcium concentration of the serum is lowered. Whether there is a phosphorus or a calcium deficiency of the diet with a lowered concentration of these substances in the serum, cod liver oil administration assists greatly in maintaining the normal concentration of these elements.

THE FATE OF CERTAIN SULFUR COMPOUNDS IN THE ANIMAL ORGANISM.

By CARL L. A. SCHMIDT and GUY W. CLARK.

(From the Department of Biochemistry and Pharmacology, University of California, Berkeley.)

Experiments were carried out to determine the fate of certain sulfonic acid compounds, with especial reference to those concerned in bile metabolism. All substances were administered by mouth to dogs kept on constant diets. Taurine is excreted in the urine unchanged and not as taurocarbamic acid as claimed by Salkowski. Cysteic acid is deaminized but the remainder of the molecule is excreted in the urine unchanged. Administration of taurocholic acid does not lead to its appearance in the urine.

Isethionic acid is excreted in the urine, no oxidation of the sulfur to sulfate taking place. These experiments indicate that dogs can neither oxidize the sulfur in sulfonic acids to sulfates nor deaminize compounds in which the amino group is in the alpha position with respect to the sulfonic acid radical.

A NEW WEDGE COLORIMETER FOR THE COMPARISON OF SOLUTIONS CONTAINING TWO COLORS, AS IN THE COLORIMETRIC pH DETERMINATION.

A DEMONSTRATION.

By VICTOR C. MYERS.

(*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York.*)

It has previously been pointed out[5] that with the use of two wedges in a modified Hellige colorimeter, it is possible to obtain all the shades of color in a given indicator from the acid to the alkaline side, when one wedge is filled with an acid solution of the dye and the other with an alkaline solution, both being made with buffer solutions of a definite pH. The utilization of the wedges of the Hellige colorimeter for this work has been under consideration for some time in this laboratory.

Barnett and Barnett[6] and Gillespie[7] have utilized essentially the same principle in the colorimetric measurement of the hydrogen ion concentration. The former authors employ a long narrow rectangular glass box having a diagonal glass partition, one being used for the acid and the other for the alkaline solution of the indicator, while the latter achieves the same result by having a small movable cup fitted over the plunger but inside the cup of a Duboscq type colorimeter.

The use of wedges which are individually movable provide a much more flexible system. The reading of the wedge containing the dominant color of the dye, *e.g.* the red in phenol red, characterizes the hydrogen ion concentration, the yellow wedge being employed simply to obtain a correct color match. This being the case it may also be employed to correct for any slight error due to extraneous yellow pigment in the unknown.

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[5] Myers, V. C., *Proc. Soc. Exp. Biol. and Chem.*, 1921–22, xix, 78.

[6] Barnett, G. D., and Barnett, C. W., *Proc. Soc. Exp. Biol. and Med.*, 1920–21, xviii, 127.

[7] Gillespie, L. J., *J. Bact.*, 1921, vi, 399.

Briefly, the colorimeter comprises a brass box 30 cm. in height, containing a rack and pinion arrangement for three wedges, the movement of the wedges being entirely within the closed box. Readings are taken from 100 mm. scales which emerge from the top of the instrument as the wedges are raised. The instrument is provided with prisms and an eye-piece in front and a milk glass plate in back for the entrance of light. For the latter a small lamp box may be substituted. A door at the side gives access to the wedges and to the cup for the unknown which is mounted on it.

With one wedge the instrument may be used as an ordinary colorimeter. The second wedge provides for bicolorimetric work, as in the pH determination. To obtain a perfect match of unknown solutions which are slightly turbid or colored a third wedge may be used.

A PRELIMINARY REPORT ON THE NEPHROPATHIC ACTION OF THE DICARBOXYLIC ACIDS AND THEIR DERIVATIVES.

By WILLIAM C. ROSE.

(*From the Laboratory of Biological Chemistry, School of Medicine, University of Texas, Galveston.*)

Comparative studies have been made of the influence upon renal function of a number of dicarboxylic acids administered subcutaneously as their sodium salts. Rabbits were used as the experimental animals. After a single dose of 1.0 to 1.5 gm. of tartrate, the preformed creatinine may increase six- or eightfold, and the blood sugar and cholesterol double in quantity, in addition to enormous increases in non-protein and urea nitrogen as previously observed by others. On the contrary, blood chlorides do not increase, and usually manifest distinct decreases in amount. Since tartrate nephritis is generally believed to involve the renal tubules primarily, the behavior of the chlorides may afford additional evidence for their elimination by the glomeruli.

In contrast to the action of sodium tartrate, sodium malate is only slightly nephropathic, and the salts of the closely related succinic and malonic acids are entirely without influence upon renal function. On the other hand, sodium glutarate (1 to 4 gm.) produces a marked retention of nitrogenous blood ingredients, accompanied by a decided fall in phenolsulfonephthalein elimination. The nephropathic action of sodium glutarate explains the observation of Baer and Blum that following its subcutaneous administration to phlorhizinized dogs, decreases occur in the excretion of urinary nitrogen, sugar, and acetone bodies.

The toxic action of sodium glutarate cannot be due to a precipitation of the calcium derivative in the renal tubules, as has been suggested in explanation of the effect of sodium tartrate, since calcium glutarate is quite soluble.

We are extending our observations to other dicarboxylic acids.

THE RELATION OF SPLENECTOMY TO GROWTH AND APPETITE IN THE RAT.

By ARTHUR H. SMITH and LEAH ASCHAM.

(From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.)

An experimental study of the alleged effect of splenectomy on the growth and appetite in the white rat was made, using the standard feeding technique in vogue in this laboratory. Observations on seventeen rats, eight of which were the progeny of splenectomized parents, gave no evidence of an increase in appetite or a variation from the normal rate of growth.

A study of the erythrocyte count on five splenectomized rats which were the progeny of splenectomized parents showed no anemia following the removal of the spleen.

CREATINURIA AND GROWTH IN THE DOG.

By VICTOR JOHN HARDING and OLIVER H. GAEBLER.

(From the Department of Pathological Chemistry, University of Toronto, Toronto, Canada.)

In studying the effect of diets varying composition upon the creatine excretion of young dogs, the authors have paid particular attention to the concomitant effect upon growth. Growth has been judged by alterations in body weight and in nitrogen balance. The change from a high protein diet to a low protein diet had previously been shown to be followed usually by a decrease in creatine excretion, though if the low protein diet were continued long enough a rise in creatine ensued. The change from a low protein diet to a high protein diet, however, is not invariably followed by a rise in creatine excretion. The creatine excretion may rise, remain constant, or fall, depending very greatly upon the growth change induced by the alteration in diet.

Changes in growth, following the introduction of an essential amino-acid into a diet in which it was probably the growth-limiting factor, are accompanied by alterations in creatine excretion. Similarly, the introduction of vitamines into a diet, in which they had previously been deficient, by causing rapid growth changes will bring about changes in creatine excretion.

THE INFLUENCE OF FASTING AND OF LOSS IN BODY WEIGHT ON THE CARBOHYDRATE TOLERANCE.

By A. I. RINGER.

(*From the Montefiore Home and Hospital, New York.*)

In this investigation, the carbohydrate tolerance of diabetic patients was studied. The plan was to determine the tolerance when the patient presented himself and to repeat these tests at intervals of years, so as to find out in what way the progression of the disease and the loss in body weight might affect it.

The patient's blood sugar was determined in the morning before breakfast. After that he was given 50 gm. of bread with one cup of weak tea. The blood sugar was again determined after the first, second, and third hours. The urine was also examined for the presence of glucose.

The same test was repeated after 1 day's fast. Invariably after 1 day's fast, the patient showed ability to utilize carbohydrate better than on the day before the fast.

These same tests were repeated on four patients who presented themselves after having undergone courses of treatment in which their body weights were reduced from 15 to 22 per cent and during which period they were kept on low caloric diets and in an aglucosuric state.

After such examinations it was found that the patients that had lost in body weight not only had no increase in their tolerance for carbohydrates, but had a decided diminution in their power to utilize carbohydrates.

AN ISOLATION OF PHENOL FROM BEEF BLOOD AND FROM HUMAN BLOOD.

By ALICE ROHDE DAVIS and ELEANOR B. NEWTON.

(From the Psychopathic Hospital, Boston.)

Phenols have been isolated from normal urine and from blood in cases of phenol injection or phenol poisoning. We have found it possible to obtain phenol as a crystalline brominated compound from both normal human blood and beef blood using mercuric acetate and sodium acetate to obtain the difficultly soluble mercurated phenols. To a protein-free blood filtrate obtained by the use of heat and acetic acid followed by colloidal iron is added mercuric acetate in sufficient amount to make a 0.25 per cent solution and subsequently sodium acetate to make a 10 per cent solution. Phenol is partially precipitated together with mercury compounds of uric acid and of combined uric acid. On concentration a further yield of mercurated phenol occurs. These compounds may be decomposed with hydrogen sulfide in acid solution or by boiling potassium iodide solution. The free phenol is then distilled from acid solution. The distillate may have the odor of phenol and gives positive tests with ferric chloride, with Millon's reagent, and with bromine water. The precipitate of brominated phenol is recrystallized from alcohol and comes down in long needles which are colorless in small amounts and melt at 92° (uncorrected). The M. P. of tribromphenol is given as 91.5°.

ANTIKETOGENESIS.

THE KETOGENIC-ANTIKETOGENIC BALANCE IN MAN AND ITS SIGNIFICANCE IN DIABETES.

By P. A. SHAFFER.

(From the Laboratory of Biological Chemistry, Washington University School of Medicine, St. Louis.)

A revision of the values[8] assigned to the ketogenic versus antiketogenic influence of protein, carbohydrate, and fat in the metabolic mixture, allows the approximate calculation of the amount of total hydroxybutyric acid produced by the catabolism of any given mixture of foodstuffs. (The main change in the values is made necessary by the fact that each molecule of glucose is ketolytic for *two* molecules of acetoacetic acid.)

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[8] Shaffer, P. A., *J. Biol. Chem.*, 1921, xlvii, 457.

The fact that the calculated expectation now approximately agrees with the actual excretion of acetone bodies in nearly all of about a dozen different subjects so far examined—normals, moderate and very severe diabetics—indicates that the underlying hypothesis is substantially correct, and that the evaluations of ketogenic versus antiketogenic influences are not greatly in error.

From these facts it follows that the *minimum* amount of food carbohydrate needed to provide a theoretical ketogenic balance can be approximately calculated for any given subject; and this figure is the absolute minimum of carbohydrate tolerance below which ketosis is *unavoidable*. The most useful formula expressing the relationship appears to be:

$$\frac{\left(\begin{array}{c}\text{Total calories of energy exchange}\\ \text{per 24 hours}\end{array}\right) - (100 \times \text{urine N})}{50} = \begin{array}{l}\text{Gm. food CH to provide}\\ \text{approximate ketogenic}\\ \text{balance.}\end{array}$$

If a diabetic has enough carbohydrate tolerance to remain "sugar-free" on a diet containing the calculated amount (or more) of food carbohydrate he will have little or no ketosis. The calculated amount allows no margin of safety against accidental increase in total metabolism from unusual activity or infection.

If the tolerance is lower the *only* recourse is to reduce the total metabolism (the amount of ketogenic material in the metabolic mixture) by rest, low protein diet, undernutrition, or fasting, until the (approximate) relationship expressed above is accomplished. For each gram of the calculated minimum of carbohydrate which is not burned (because of low tolerance or because not available in food) slightly more than a gram of total hydroxybutyric acid is excreted.

A COMPARISON OF ACETONURIA CAUSED BY DISEASE WITH THAT CAUSED BY DIETS LOW IN CARBOHYDRATE.

By ROGER S. HUBBARD, SAMUEL T. NICHOLSON, Jr., and FLOYD R. WRIGHT.

(From The Clifton Springs Sanitarium, Clifton Springs, N. Y.)

Mathematical ratios were proposed to express the molecular relationship between glucose and fat for any diet fed. For normal cases receiving enough food to maintain metabolic equilibrium the ratio was based on the foods ingested; for diabetics not in metabolic equilibrium the ratio was based on the foods burned in the body, as calculated from the basal metabolism, carbohydrate ingested, and sugar and nitrogen excreted. Charts were shown in which the values of these ratios were compared with the acetone excretion, and a similarity of response was shown in the two conditions. From a study of the values of the ratio which corresponded with a very slight increase of the acetone excretion above normal, it was concluded that the glycerol radical of the fats probably figures as a source of antiketogenic material to the extent to which it will yield glucose when fed.

STUDIES OF THE METABOLISM OF DIABETES.

By RUSSELL M. WILDER, WALTER M. BOOTHBY, and CAROL BEELER.

(From the Mayo Clinic and Foundation, University of Minnesota, Rochester.)

A chart is presented giving the more important data of feeding experiments in a case of diabetes of great severity. These experiments were designed to throw light on the rôle of ingested protein on the diabetic metabolism. Observations were continued for 11 weeks with daily determinations of the urinary excretion of nitrogen, sugar, ammonia, acetone bodies, total acids, and phosphates and frequent determinations of the carbon dioxide-combining power of the plasma, the sugar, acetone, and fat content of the blood and the respiratory metabolism.

The following conclusions are submitted:

1. The postabsorptive or *basal* metabolic level of the diabetic individual is materially affected by the previous diet. In the

undernourished patient it may be found as low as 32 per cent below the Du Bois standard normal for a healthy person of like age, sex, and surface. The ingestion of food containing 1 gm. of protein per kilo of body weight with fat and carbohydrate in such an amount that the daily maintenance energy requirements of the patient were exceeded, caused an elevation of the basal level. The ingestion of 3 gm. of protein per kilo of body weight per day caused a greater rise in the basal metabolic rate than occurred with isocaloric amounts of other foods. A cumulation of the specific dynamic action of protein seemed to account in the main for the elevation of the *basal* metabolic level which occurred.

2. The rate of sugar utilization of this diabetic individual was depressed by high calorie diets, being much more markedly depressed by protein than by isocaloric amounts of fat. This protein effect was not primarily due to the sugar and ketogenic substances, which the ingestion of protein throws upon the metabolism, but to some other more specific action of protein.

3. Throughout this series of experiments the rate of sugar utilization varied inversely with the basal metabolic level of the patient, rising as the basal metabolic rate fell, and *vice versa.* This behavior suggests a definite interrelationship of the two and the fact that ingested protein elevates the *basal* metabolic rate makes it seem possible that it is by this mechanism that it adversely affects the rate of sugar utilization.

4. Diets relatively high in fat but low in protein, and planned to contain nearly 2 gm. molecules of fatty acid to 1 of glucose on two occasions checked and controlled a dangerous and rising acidosis. The data of the metabolism of 2 days when acidosis was minimal indicated that on these days the actual ketogenic-antiketogenic ratio in the metabolizing mixture was 1.53 and 1.78 respectively. It is suggested that the proportion of fatty acid which will completely burn with a limited amount of metabolizing glucose is not the same at all basal metabolic levels, but may be increased by measures designed to depress the basal metabolic rate.

THE EFFECT OF LOSS OF CARBON DIOXIDE ON THE HYDROGEN ION CONCENTRATION OF URINE.

By E. K. MARSHALL, Jr.

(From the Department of Physiology, the Johns Hopkins University, Baltimore.)

In certain conditions the escape of carbon dioxide from urine may have a marked effect in lowering the hydrogen ion concentration. Concentrated acid urines as ordinarily obtained on a mixed diet do not change appreciably in hydrogen ion concentration when shaken with air although practically all of the carbon dioxide may be given off. When very dilute urines or neutral and alkaline ones obtained after administration of alkali are so treated, there is a distinct decrease in acidity or increase in alkalinity. Precautions similar to those used in the case of blood are necessary in determining the pH of these urines. Experiments on man and animals indicate that the lowest hydrogen ion concentrations reported for urine may be too low due to neglect of proper precautions to avoid the loss of carbon dioxide.

A STUDY OF THE METABOLISM AND THE RESPIRATORY EXCHANGE IN POULTRY DURING VITAMINE STARVATION.

By R. J. ANDERSON and W. L. KULP.

(From the Biochemical Laboratory, New York Agricultural Experiment Station, Geneva.)

In an endeavor to obtain some information regarding the changes which occur in the metabolism of an animal which is deprived of the water-soluble vitamine B, a series of experiments was made on poultry. This study included observations under normal conditions of diet and then a state of vitamine starvation was induced by feeding polished rice until polyneuritis was finally produced.

A diet of polished rice caused a loss of appetite in our experimental animals and the food consumption fell to a low level. The continued lack of vitamine B in the diet caused a serious impairment of the digestive functions which during polyneuritis resulted in a practical cessation of digestion and assimilation.

During vitamine starvation there was a decided fall in the intensity of the metabolism corresponding to the decreased food consumption. The basal heat production in some cases fell to more than 50 per cent under that of the normal basal metabolism and the respiratory quotients averaged from 0.73 to 0.80 about 18 hours after feeding. Respiratory quotients approaching unity were obtained, however, during the first 3 or 4 hours after feeding polished rice until a short time before active symptoms of polyneuritis developed.

But during polyneuritis, although the crop of the fowl contained much undigested rice, the respiratory quotient seldom rose above 0.75, indicating a nearly complete inability at that time to utilize this food.

HEAT ELIMINATION AND GASEOUS EXCHANGE IN GRAPEFRUIT DURING STORAGE.

By C. F. LANGWORTHY and H. G. BAROTT.

(From the Office of Home Economics, States Relations Service, United States Department of Agriculture, Washington.)

The experiments here described belong to the same general series as those reported in 1919[9] for bananas and apples and in 1920 for celery and eggs, and like the earlier ones were carried out in the large respiration calorimeter of the Office of Home Economics. In the present case the tests, which were conducted December 2 to 10, 1920, were made with grapefruit such as are imported in large quantities from Porto Rico. Refrigerator ships cannot be obtained for this trade, the fruit is transported in the holds of ordinary vessels where there is no regular ventilation and the temperature averages from 75 to 80°F., and the loss through decay, molds, and similar causes is very great. The experiments were made at the request of and in cooperation with the Porto Rico Experiment Station in the hope that accurate observations of the heat elimination and gaseous exchange of the fruit under conditions comparable to those on shipboard might yield information of practical service to shippers.

123 kilos of commercial pack grapefruit were used. Single layers of these were placed in the calorimeter on shelves made of

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[9] Langworthy, C. F., Milner, R. D., and Barott, H. G., *J. Biol. Chem.*, 1920, xli, p. lxix.

¼ inch galvanized mesh wire and set 1 foot apart. Differential thermocouples were distributed among the fruit so that its temperature could be determined relative to its surroundings and resistance thermometers made it possible to determine the temperature of both the air within the calorimeter and of the walls. When the fruit had been placed in the chamber, the latter was sealed, the circulation of gases started, and the temperature adjusted to 78°F., at which point it was kept throughout the 8 days of the experiment. Determinations of the gaseous exchange and heat elimination were made at approximately 24 hour intervals, each determination representing the mean value for the period just ended.

The heat eliminated varied from 1.7 to 2.6 calories per hour for the total weight of fruit. The carbon dioxide elimination varied from 1.8 to 2.4 gm. per hour. The amount of water vapor given off was also very uniform, varying from 23.8 to 25.6 gm. per hour. The oxygen absorbed showed more variation and ranged from 0.7 to 3.1 gm. per hour. This irregularity in the oxygen may be accounted for by the fact that the oxygen is determined by difference and not measured direct as is the carbon dioxide and that with such small quantities of oxygen, a slight error in the residual analysis due to the large amount of moisture eliminated might appear as a large percentage error in the oxygen figure.

Since the heat elimination even under these experimental conditions with excellent ventilation and constant removal of heat, was sufficient to keep the temperature of the fruit above that of the surrounding air, it seems reasonable to suppose that where there is no ventilation, as in the hold of many ships carrying grapefruit, the temperature of the fruit would ultimately become several degrees higher than when it was placed on board.

A METHOD FOR THE DETERMINATION OF LACTIC ACID IN BLOOD.

By E. L. SCOTT and F. B. FLINN.

(From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York.)

The method which we describe is based upon that described by Ryffel. We have so modified the method that the filtrate obtained in Folin and Wu's system of blood analysis is used.

We have obtained satisfactory results in using the filtrate from 5 to 20 cc. of blood.

An aliquot of the filtrate is evaporated to about 40 cc. in an evaporating dish upon the water bath or if less than this, it is placed directly in a 500 cc. distilling flask with side neck and enough water added to bring it up to 40 cc. It is then neutralized with solid Na_2CO_3 and 45 cc. of concentrated sulfuric acid are added drop by drop, the flask being held under running water to keep it from heating. The flask is then connected with a Liebig condenser and a steam generator and distilled with a current of steam for 30 minutes, or until 150 cc. of distillate have passed over. The receiver is immersed in ice water. In this distillation the temperature of the contents of the flask is raised rapidly to 155°C. and kept between 155 and 158°C. during the entire process.

The distillate is then neutralized with 2 per cent NaOH solution and redistilled into an Erlenmeyer flask which contains 20 cc. of a 6 per cent solution of KOH and 20 cc. of 0.04 N standard iodine solution. This flask is also immersed in ice water. When 100 cc. have distilled over, the flask is removed and slowly warmed to about 20°C. 20 cc. of 15 per cent HCl are then added and the contents titrated with 0.04 N standard thiosulfate solution.

If care is taken to prevent heating during the addition of the sulfuric acid and to keep the temperature during the first distillation within the range indicated, the results are very concordant.

The thiosulfate solution should be standardized by treating a known amount of lactic acid in exactly the same manner as that described for the Folin and Wu filtrate.

THE BLOOD SUGAR CONTENT OF CAPILLARY BLOOD AS COMPARED WITH THAT OF VENOUS BLOOD.

By ISAAC NEUWIRTH AND ISRAEL S. KLEINER.

(From the Department of Physiological Chemistry of the New York Homeopathic Medical College and Flower Hospital, New York.)

Twenty normal students were employed as subjects. Blood was obtained simultaneously from a finger and from a vein of the same arm and was analyzed by the micro method of one of the authors (K). In eighteen out of the twenty cases the results did not differ by more than 0.01 per cent and in the other two by only 0.02 per cent. The average of all twenty is 0.136 per cent for the capillary and 0.130 per cent for the venous blood. The method was also compared with a macro method and the conclusion is reached that the analysis of capillary blood by this micro method gives results which, for clinical purposes, can be considered identical with the venous blood sugar.

THE CALCIUM AND MAGNESIUM CONTENT OF THE HUMAN FETUS.

By MAURICE H. GIVENS and ICIE G. MACY.

(*From the Research Laboratories, Western Pennsylvania Hospital, Pittsburgh.*)

Twenty-five fetuses, ranging from 90 to 400 mm. in length and consequently varying from 3 to 8 lunar months of age, have been dried and ashed. The ash has been analyzed for its content of calcium and magnesium.

The increase in length of the body appears to be at a gradual rate at all times. The total solids of the body increase gradually and slowly up to the fifth month, thereafter at a more rapid rate; likewise is this true of the ash and its calcium content. The magnesium figures do not become significant until about the seventh month.

Up to the third month the calcium requirement of the fetus approximates *in toto* 300 mg. of CaO and 60 mg. of MgO. From this time up to the eighth month, the demand for lime is much greater, relatively and absolutely. The magnesium content of the body varies considerably. At no time does it equal or even approximate the amount of calcium present in the body.

The total ash varies from 3.85 to 33.4 per cent of the dry body weight. Calcium oxide represents from 1 to 12 per cent of the dry body weight and from 24 to 50 per cent of the total ash. In the majority of cases MgO is less than 1 per cent of the dry body weight and from 3 to 15 per cent of the total ash.

For the first 3 or 4 months of pregnancy the fetal demand for CaO varies from 10 to 30 mg. per day, whereas for the total period of pregnancy it may average 100 mg. per day. Whether or not the total lime requirement of the fetus can be met from the mother's food intake remains to be investigated.

THE EFFECT OF CHANGES IN THE PROTEIN AND ENERGY OF THE DIET OF MILKING COWS UPON THE MILK YIELD AND UPON THE AMINO N OF THE BLOOD.

By C. A. CARY.

(From the Research Laboratories of the Dairy Division of the United States Department of Agriculture, Beltsville.)

In five experiments on cows the changes in the blood by which changes in either the protein or carbohydrate of the diet affect the yield and composition of milk were investigated. The animals were put on approximately adequate rations. In two experiments the energy was reduced 33 per cent; in one of these the protein also was cut 50 per cent and in the other it was unchanged. In two other experiments the protein was cut 50 per cent; in one the quality of the diet protein was also reduced and in the other it was kept constant. In the fifth experiment only the quality of the diet protein was varied. In the first four experiments the cows were finally put back on the original rations. The amino N of the blood and plasma and the yield and composition of the milk were followed. The results may be summed up as follows:

1. The cut in the amounts of energy or protein or both in the ration reduced the milk yield and the concentration of milk N. The concentration of lactose was unchanged, and that of milk fat was reduced only when the protein alone was reduced. The amino N of the blood and plasma was reduced except when the energy of the ration was unchanged and the quality of the diet protein was also reduced. Reducing the quality of the diet protein either with or without a reduction in quantity reduced the milk yield without reducing the amino N of the blood and plasma.
2. When these changes in diet were reversed the changes in the yield and composition of the milk were reversed, but these changes were not the exact reciprocals of those produced by lowering the rations. Only when the amount of protein was alone increased without any change of quality did the amino N of the blood rise. It was, in general, at first reduced.

The results indicate that changes in either the carbohydrate or protein of the diet affect the yield and composition of milk through changes in the quality and quantity of the amino-acid mixture of the blood, and that the quality of the diet protein is an important factor in effecting these changes.

ACID-BASE METABOLISM IN INFANTS.

By ALFRED T. SHOHL.

(From the Department of Pediatrics, Yale University School of Medicine, New Haven.)

A new principle in the study of acid-base metabolism is presented. The food and excreta are analyzed for all the acids and all the bases. Their acid or base value is calculated in cc. of normal solutions. From the value of the intake is subtracted the value of the output. The remainder represents the balance. If the body is in equilibrium the balance is zero, for the output equals the intake. In acidosis, if base is excreted, it represents acid retention or abnormal acid production. If there is an acid excretion it represents a retention of base by the body. In infants of 1 year the retention is roughly 100 cc. 0.1 N base per day.

The acid-base value of the urine can also be determined by titration according to Palmer and Henderson. The acid excretion equals the free acid plus ammonia. However, if organic acid is present this must be determined and subtracted. In alkaline urines the carbonates must also be determined and subtracted. For as the organic acids bind base so the carbonates too unite with base and, therefore, represent base excretion. The carbonates, therefore, act as buffers and preserve neutrality in alkaline urines. Unless these corrections are made the acid-base value of the urine cannot be determined by titration.

A method is presented for the titration of the acid-base value of the stools. The principle is to determine the organic acid by extraction. The stool is then titrated in alcoholic solution and the acid-base value determined by subtraction. If carbonates are present they must be subtracted as in the case of the urine. In the stools the buffer value of the phosphates is unimportant. The neutrality is preserved by the fatty acids and soaps and by the carbonates.

RESPIRATORY QUOTIENT STUDIES IN SCURVY AND BERI-BERI.

By H. J. GERSTENBERGER and C. W. BURHANS.

(From the Department of Pediatrics, School of Medicine, Western Reserve University, Cleveland.)

This study, begun during the close of 1916, was started with the idea of bringing evidence for or against the theory that scurvy was the result of a disturbed carbohydrate metabolism, caused, owing to the absence of or the reduction in the amount of antiscorbutic vitamine in the diet, by the establishment of a disproportion between the quantity of the antiscorbutic vitamine in the food on the one hand and its carbohydrate content on the other. The relative scarcity of scorbutic infants and early difficulties in getting scorbutic guinea pigs to take adequate amounts of food at the desired time was responsible for the inclusion of beri-beri in this study, a disease which according to Funk can be produced with greater rapidity by increasing the carbohydrate intake of the pigeon.

Whenever the opportunity was offered scorbutic infants were studied. Finally, by using Kellers malt soup, it was possible to establish satisfactory experimental conditions regarding scurvy and food intake in guinea pigs and to study the respiratory quotients in these animals.

The results of these studies bring evidence that scorbutic infants and guinea pigs, as well as polyneuritic pigeons can burn carbohydrates completely, and that, therefore, the study of respiratory quotients of animals ill with scurvy or beri-beri brings no evidence in favor of the correctness of the above mentioned theories.

CHEMICAL BLOOD CHANGES IN PNEUMONIA.

By JOHN A. KILLIAN.

(From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York.)

These studies comprise determinations of the non-protein and urea nitrogen, uric acid, creatinine, sugar, chlorides, and carbon dioxide-combining power of the blood in cases of pneumonia of various types. In some instances, also, parallel estimations of the total nitrogen and chlorides of the urine have been made. A significant rise in the undetermined nitrogen of the blood has been noted about the period of the resolution of the pneumonia. Consequent to this, an impairment of kidney function develops, resulting in an accumulation of the nitrogenous waste products in the blood. The rise in the non-protein nitrogen is followed by an increase first, in the uric acid; later, in the urea nitrogen; and finally, in the creatinine. In fatal cases a rise of the creatinine to more than 4 mg. per 100 cc. was noted. An attempt has been made to correlate the changes in the chloride concentration and the carbon dioxide-combining power of the blood throughout the course of the pneumonia with the clinical manifestations of the disease.

FURTHER STUDIES ON THE REACTION OF DYING TISSUES.

By WITHROW MORSE and R. GOLDBERG.

(From the Laboratory of Physiological Chemistry, West Virginia University, Morgantown.)

It has been reported by others and by the authors that as tissue dies, the low alkalinity passes to a relatively high acidity. Preliminary experiments showed that this reaction takes place with great rapidity, and Morse and van der Heyde have reported the results of a study of mammalian liver using the gas chain method for estimating acidity. In this study the concentration of hydrogen ions within a few moments after excision of the liver was found to be in the neighborhood of pH 4.6. Criticism had been directed against these results since there must be time given for equilibrium to be reached, and in order to rule out this difficulty we have repeated the experiments, with modified technique, using the Sörensen colorimetric method upon ice-cold alcoholic extracts, following somewhat the technique of Fletcher and Hopkins in their work on lactic acid in muscle. The same average results were obtained in this procedure as we obtained with the electrometric method. Attention was then turned to lactic acid as being responsible for the sudden development of acidity. A basal figure for lactic acid estimated as zinc lactate by the zinc oxide method was found to be 0.035 gm. per cent wet weight of tissue. This figure approaches that obtained by Fletcher and Hopkins and more recently by Foster and Moyle for basal lactic acid in frog muscle. This concentration of lactic acid is adequate to explain the acid figures. Kidney tissue gives similar acid figures.

CALCIUM METABOLISM IN TETANY.

By FRANK P. UNDERHILL, WILDER TILESTON, and
L. JEAN BOGERT.

(From the Department of Pharmacology and Toxicology, Yale University, New Haven.)

When compared to normal individuals under the same experimental conditions a subject with tetany, presumably of gastrointestinal origin, showed a normal type of behavior to calcium intake except that there was evidence of a greater tendency to store calcium temporarily on a calcium-rich diet. On the other hand, on a calcium-poor diet this stored calcium is eliminated to a much greater extent than occurs in the normal subject. These facts may, perhaps, be interpreted to mean that the organism with tetany shows a greater need for calcium than the normal individual but that in tetany the regulation of calcium equilibrium is in an unstable condition.

INDOLETHYLAMINE IN THE URINE OF PELLAGRINS.

By M. X. SULLIVAN.

(From the Hygienic Laboratory, Washington.)

About 40 liters of mixed urine of pellagrins were treated with lead acetate and with basic lead acetate and the filtrate, freed from lead, was concentrated to 1,500 cc. The concentrated solution, made 5 per cent acid with H_2SO_4, was treated with phosphotungstic acid in 5 per cent H_2SO_4. In the filtrate from the resulting precipitate, indolethylamine was found. The solution, freed from phosphotungstic acid by baryta and from barium by dilute H_2SO_4, was concentrated *in vacuo* to 300 cc. and filtered. The filtrate was strongly acidified with concentrated HCl and filtered from a precipitate which formed. The filtrate, concentrated to 100 cc., was treated with 95 per cent alcohol and filtered. The filtrate, freed from alcohol, was treated with picric acid. A dark red precipitate of long needles occurred which, on recrystallization from acetone, had a melting point of 242°C. From the picrate a chloride was obtained, melting at 244–245°. A small amount of free base was likewise obtained of which the melting point was not obtained. The free base and syrup, however, gave an intense violet-blue color with glyoxylic and sulfuric acids as did the hydrochloride, likewise. From the color, crystalline shape, solubility, and melting point of the picrate, the melting point and the Hopkins and Cole's reaction of the chloride, and the Hopkins and Cole's reaction of the free base, it is evident that the compound is indolethylamine.

THE CHICK AS AN EXPERIMENTAL ANIMAL IN VITAMINE STUDIES.

A PRELIMINARY REPORT.

By A. D. EMMETT and GAIL E. PEACOCK.

(*From the Biological and Research Department, Parke, Davis and Company, Detroit.*)

Four series of feeding trials were carried out using young White Leghorn chicks in comparison with rats and pigeons. It was found that in modifying the rations for the chicks by increasing the nitrogen, introducing some roughage, and supplying a liberal amount of charcoal, oyster shell, and grit, that the birds were seemingly well adapted to studies where either the vitamine A or B was absent.

The chicks were all fed for the first 2 weeks after hatching in the most approved practical manner so as to tide them over the initial critical period of growth. They were then placed in confinement and fed on the same ration for 7 to 10 days when they were given the various experimental diets. The chicks fed upon the normal control diet grew and gained at the usual rate and had the appearance of healthy fowl. Those fed upon the vitamine B minus diet soon showed definite signs of the deficiency—ceasing to grow, losing in weight, and developing other symptoms of malnutrition, culminating in polyneuritis in 10 to 12 days. Hydropericardium, hypertrophy of the adrenals and gall bladder, and atrophy of testes were found on autopsy. The chicks fed the vitamine A minus ration continued to gain normally for a longer time than did those on a lack of the vitamine B. They showed a weakness of legs; poor condition of the feathers; fading or bleaching of the pigment in the legs, bill, and toes; anemia; edema about the eye, followed by an ophthalmic condition similating xerophthalmia; beading of the ribs; atrophy of the testes, and hypertrophy of the adrenals and gall bladder. The period of duration was from 14 to 21 days. In the case of the vitamine C, the requirements of the chick appear to be much less than for vitamines A and B.

The advantages in favor of the chicks are: ease of obtaining large numbers of animals; the minimum amount of attention and lessened cost compared with the breeding of the rat; the greater uniformity of the animals and hence lessened degree of variation; and a shortening of the time and duration of the feeding tests, due to the apparent increased sensitiveness of the chick to the lack of the vitamines.

The possibilities of the chick in such feeding trials appear to suggest that they are both promising and practical.

CALCIUM IN EGG-SHELL FORMATION.

By G. D. BUCKNER, J. H. MARTIN, W. C. PIERCE, and A. M. PETER.

(*From the Kentucky Agricultural Experiment Station, Lexington.*)

Six lots of 7 month old White Leghorn pullets were fed for 8 months, restricted as follows:

Lot 1. Grains + tankage + no mineral material.
" 2. " + " + granite grit, *ad libitum*.
" 3. " + " + " " " " + oyster shell, *ad libitum*.
" 4. " + " + " " " " + limestone " "
" 5. " + " + limestone, " "
" 6. " + " + rock phosphate, *ad libitum*.

Tankage (12.5 per cent of total feed) containing 6.4 per cent P_2O_5 was fed in the mash. The grit used contained 2.42 per cent CaO soluble in strong HCl.

Results obtained from the analyses of the leg bones and the egg-shells and the number of eggs produced in each lot indicate that the calcium carbonate can be utilized by the hen for the production of egg-shells and bones but that the calcium in tricalcium phosphate can only be utilized for the growth of bones and not for egg-shell production. Also that calcium starvation is not the determining factor in the production of shell-less eggs.

THE HEAT OF ENZYME REACTION.

A STUDY OF THE HEAT PRODUCED IN THE CATALASE REACTION.

By SERGIUS MORGULIS.

(From the Department of Biochemistry, College of Medicine, University of Nebraska, Omaha.)

In a previous paper on the catalase reaction[10] as well as in the publications of earlier investigators the fact has been noted that the velocity constant of the reaction in the first few minutes diminishes very rapidly. It seemed probable that these changes are due to temperature alterations, and the experiments here reported were performed to test this hypothesis. The results demonstrate that the catalase reaction is exothermic, and that it is accompanied by a definite heat production.

The experiments were made with a preparation of liver catalase. The volume of the mixture and its reaction ($pH = 7$) were kept constant in all tests. Only the relative amounts of catalase and hydrogen peroxide varied. The reaction took place in a thermos bottle, and the rise in temperature was measured with a special thermometer. Experiments with boiled solutions of catalase showed that only a negligible heat production (0.02°C. per minute of shaking) occurs which must be used as a correction in the experiments with the unboiled catalase. Without discussing the weak points of the technique, the heat production in the catalase reaction is now an established fact, as is shown by the data recorded below. The curves of heat evolved differ in no essential respect from curves plotted from data of oxygen set free in the reaction. The maximum heat production occurs in the first 5 minutes; *i.e.*, during the period when the velocity constant changes most abruptly. It is, therefore, concluded that the abnormalities in the reaction velocity are directly associated with the thermal phenomena accompanying the catalase reaction. The heat production is evidently conditioned upon the intensity of the reaction, and by further refinement of the apparatus it is expected that it will be possible to follow the progress of the reaction by the temperature changes not only for catalase but for other enzymes as well.

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[10] Morgulis, S., *J. Biol. Chem.*, 1921, xlvii, 341.

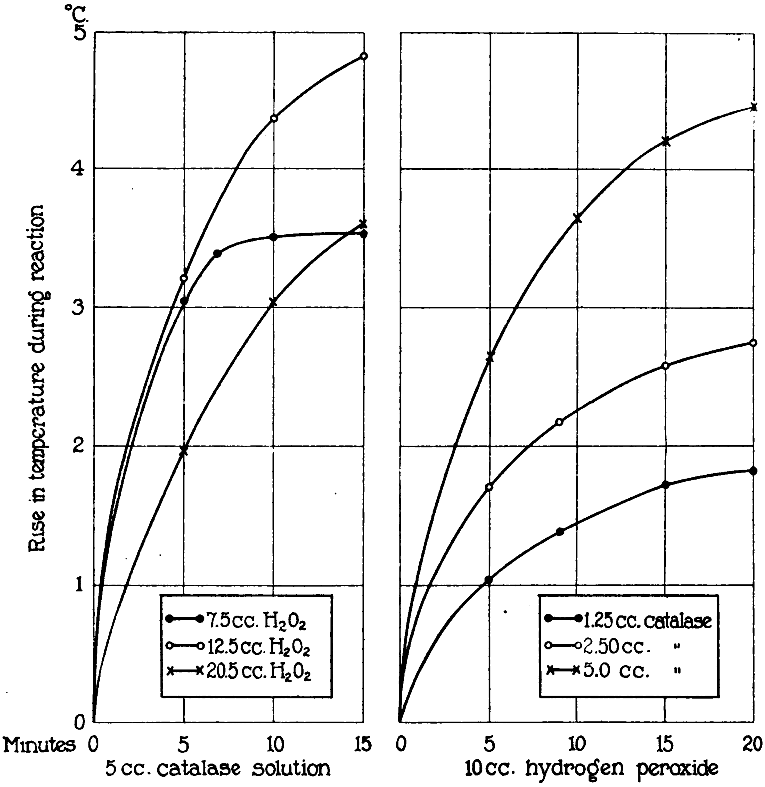

Fig. 1.

FURTHER EXPERIMENTS ON THE PREVENTION OF RICKETS IN RATS BY EXPOSURE TO LIGHT.

By ALFRED F. HESS.

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.)

The development of rickets in rats fed the rachitic diet described by Sherman and Pappenheimer, can be prevented by daily exposures to direct sunlight for 15 minutes. Sunlight which has traversed flint window glass loses this potency. After having been reflected from a white surface it retains some of its effectiveness. Rickets, likewise, can be prevented by exposure for about 2 minutes to the rays from a mercury-vapor lamp. The ozone emanations from this lamp have no protective value. Rays from an open carbon arc lamp protect. Soft x-rays are ineffective.

Prolonged exposure to direct sunlight failed to prevent or to delay the onset of scurvy in guinea pigs.

GROWTH AND REPRODUCTION IN RATS ON A MILK DIET.

By H. A. MATTILL.

(From the Department of Physiology, University of Rochester, Rochester.)

The failure of adolescent growth and of reproductive ability in female rats on whole milk powder was not corrected by diluting the milk powder with lard, starch, and salts in varying proportions. The animals still showed the characteristic slowing of growth rate at adolescence and were infertile. Males grew normally but their reproductive efficiency was questionable. Additions of protein-free milk, of cod liver oil, or of traces of KI after the animals had reached the age of 150 to 175 days did not result in fertility or renewed growth. Ordinary stock rat food did not do it. When animals that grew up on stock food were transferred to milk rations at about the age of adolescence they likewise failed to rear their young, or were sterile.

The addition of a small amount of yeast to milk rations caused the females to cast litters regularly and repeatedly, but none ever lived more than a few days.

Satisfactory correlation between fertility and weight of gonads is not yet possible because the variability is too large for the number of animals thus far observed.

Of 16 males on milk rations	8 had average testes weights varying + 16 per cent from normal and
	8 had average testes weights varying − 42 per cent from normal.
Of 31 females on milk rations	6 had average ovary weights varying + 16 per cent from normal and
	25 had average ovary weights varying − 36 per cent from normal.

The plus figures are undoubtedly within the range of normal variability; the minus figures may not be. The partial success induced by yeast addition did not materially alter this distribution. Underdeveloped testes seldom, if ever, contained spermatozoa and histological material is now being studied.

RIGOR MORTIS.

By J. B. COLLIP.

(From the Department of Pathological Chemistry, University of Toronto, Toronto, Canada.)

When muscle plasma is carefully acidified, a precipitate of the proteins is brought about. This precipitate redissolves on the further addition of acid and reappears when alkali is slowly added, and again redissolves on the further addition of alkali. If one filters at the point of maximal precipitation the pH of the filtrate falls between 6.3 to 6.6 index. This corresponds to the pH index of muscle going into rigor. It is, therefore, suggested that the onset of rigor is due to physical changes, the development of post-mortem acidity in the tissue resulting in rigor when the isoelectric point of the proteins is reached. The passing of rigor may be associated both with the development of further acidity and with enzymatic action.

STUDIES OF THE NORTH AMERICAN SARRACENIACEÆ.

By JOSEPH S. HEPBURN, E. QUINTARD ST. JOHN,
FRANK M. JONES, and WILLIAM F. BAKER.

(*From the Constantine Hering Laboratory, Hahnemann Medical College, Philadelphia.*)

Liquor from closed pitchers of *Darlingtonia californica* contained diastase; protease, maltase, emulsin, invertase, and urease were absent. Liquor from closed pitchers of *Sarracenia flava* contained invertase and lipase; maltase, emulsin, diastase, urease, and esterase were absent; it had a surface tension of 66.4 dynes per cm. Protease occurred in the liquor from closed pitchers of the *Sarraceniæ*. Liquor from closed pitchers was bacterially sterile, that from open pitchers contained proteolytic bacteria. The pitchers absorbed nutrient compounds from their cavities. The pitcher liquor of the *Sarraceniæ* produced permanent cessation of motion and caused ants to sink more frequently and more promptly than in water. The liquor did not hemolyze human erythrocytes; that from closed pitchers had no toxic action when injected into the lymph sac of frogs or subcutaneously into a guinea pig. Prolonged administration of the mother tincture of either the rhizomes or the pitchers of *Sarracenia minor, Sarracenia flava, Sarracenia drummondii,* or *Sarracenia rubra* to rabbits by mouth in small doses produced allopecia, formation of red punctate umbilicated papules, rupture of the postules, formation of crusts, and other symptoms. The rabbits recovered when medication ceased, and had then acquired an immunity to the action of the drug. The rhizomes did not contain protease.

CHANGES IN THE REFRACTIVE INDEX OF THE BLOOD SERUM OF THE ALBINO RAT WITH TEMPERATURE.

By F. S. HAMMETT and IDA S. TELLER.

(*From The Wistar Institute of Anatomy and Biology, Philadelphia.*)

A study of the changes in the refractive index of the blood serum of the albino rat with rising temperature showed that two types can be differentiated according to the nature of the response. In the first type the changes in the refractive index coincide with those of the solvent water and can be attributed to this serum constituent. In the second type, the curve of the changes of refraction with rising temperature falls away from that of water. This demonstrates a participation in the response of serum constituents other than that of the solvent water. The factors which contribute to this difference are unknown, although there is a possibility that a seasonal variation may be a determinant. It is certain that in this series the factors of body length, body weight, age, water content of serum both before and after the experiments, and previous state of digestion and absorption are not the causes of the difference between the two groups.

The correction for the reduction of the observed angle of refraction to the common base at 20° when readings are taken at different temperatures is obtained by the use of the formulas;

$$I = i - 1.25' \ (t-20) \text{ and}$$
$$I = i + 1.25' \ (20-t)$$

where I is the corrected angle of refraction; i, the observed angle of refraction; t, the observed temperature; and 1.25′, the change in minutes for each degree of change in temperature. These formulas hold for temperatures between 17.5 and 35°C.

TOTAL METABOLISM IN EXOPHTHALMIC GOITER.

By WALTER M. BOOTHBY and IRENE SANDIFORD.

(From the Section of Clinical Metabolism, Mayo Clinic and the Mayo Foundation, University of Minnesota, Rochester.)

The work included a quantitative study of the food intake, urinary elimination, the blood chemistry, and the respiratory metabolism in three cases of exophthalmic goiter. The total metabolism was found to be frequently in excess of 5,000 calories per day and occasionally over 6,000 calories, which is in marked contrast to the daily food ration of 1,500 to 1,800 calories common in many countries during the war.

THE METABOLISM OF INORGANIC SALTS.

By ERWIN G. GROSS and FRANK P. UNDERHILL.

(From the Department of Pharmacology and Toxicology, Yale University, New Haven.)

In normal dogs the relationships existing between calcium, magnesium, potassium, sodium, etc., in the blood are remarkably constant under fixed experimental conditions. When changes occur in one direction an immediate compensation occurs.

THE APPARENT ACID DISSOCIATION CONSTANTS OF OXYHEMOGLOBIN AND REDUCED HEMOGLOBIN.

By EDWARD A. DOISY, A. P. BRIGGS, and K. S. CHOUKE.

(From the Laboratories of Biological Chemistry, Washington University, School of Medicine, St. Louis.)

Determinations were made of the distribution of base between oxyhemoglobin or reduced hemoglobin and carbonic acid. From these data, the apparent dissociation constants of the two forms of hemoglobin were calculated.

The isoelectric points of oxyhemoglobin and reduced hemoglobin were determined by equilibrating the hemoglobin-base solutions with high tensions of carbon dioxide. The point at which the combined carbon dioxide equals the inorganic base of the solution was taken as the isoelectric point.

By plotting the bicarbonate values against pH, the relationship of loss of oxygen to increase of bicarbonate at a constant pH was obtained. The magnitude of these values was of the same order as those obtained on defibrinated blood. The loss of approximately 2 volumes of oxygen allows the blood to take up 1 volume of carbon dioxide without a change of hydrogen ion concentration.

COLORIMETRIC METHODS FOR THE DETERMINATION OF HOMOGENTISIC ACID AND MAGNESIUM.

By A. P. BRIGGS.

(From the Laboratories of Biological Chemistry, Washington University School of Medicine, St. Louis.)

Phosphomolybdic acid is especially susceptible to reduction by *p*-diphenols. Advantage of this reaction has been taken, first, to determine homogentisic acid in alcapton urine, and again to determine magnesium by the phosphorus content of the $MgNH_4PO_4$ precipitate.

ON THE RELATION OF THE HYDROGEN ION CONCENTRATION TO THE FERTILIZATION OF MARINE EGGS.

By G. H. A. CLOWES and HOMER W. SMITH.

(From the Biochemical Research Laboratory, Eli Lilly and Company, Indianapolis.)

The permeability of sea urchin and starfish eggs to homologous sperm varies with the hydrogen ion concentration of the sea water, regardless of whether the acid involved is carbonic acid or a mineral acid. In each species there is a hydrogen ion concentration at which the permeability to sperm is greatest, and another at which the permeability is zero. This indifference to the nature of the acid employed exhibits a striking contrast to the results on cell division reported in a preceding paper.[11]

ON PHYSICAL AND CHEMICAL VARIATIONS IN THE COMPARISON OF INTERIOR AND SURFACE PROTOPLASMA.

By G. H. A. CLOWES, ROBERT L. CHAMBERS, and IRVINE PAGE.

(From the Biochemical Research Laboratories, Eli Lilly and Company, Indianapolis.)

By comparing the relative cytolytic effects exerted by saponin and digitonin on the one hand, and hypotonic solutions on the other, it was possible to demonstrate that the surface of marine eggs contains a larger proportion of cholesterol than does the interior, or cholesterol is present in a more effective form as a protective agent against the action of saponin, digitonin, etc.

SOURCES OF ERROR IN THE DETERMINATION OF CHLORIDES IN BLOOD.

By ISIDOR GREENWALD.

(From the Harriman Research Laboratory, Roosevelt Hospital, New York.)

In the determination of chlorides in blood, it is usual to precipitate the protein by some acid precipitant. It is generally assumed that the precipitating anion completely displaces the chlorine ion from combination with protein. This is not always the case, particularly when nitric acid or metaphosphoric acid is used.

If picric acid is used as the precipitant, the presence of a purine, apparently hypoxanthine, in the cells makes the results too high unless about 20 per cent nitric acid is present. The precipitate of hypoxanthine—silver-picrate—contains much more silver than the molecular formula given in the literature would require.

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[11] Clowes, G. H. A., and Smith, H. W., *J. Biol. Chem.*, 1922, l, p. iv.

A MICRO METHOD FOR THE DETERMINATION OF UREA IN BLOOD.

By ISRAEL S. KLEINER.

(From the Department of Physiological Chemistry of the New York Homeopathic Medical College and Flower Hospital, New York.)

The method consists in direct Nesslerization of a Folin-Wu filtrate after the urea has been digested by urease. The color is compared, in the Klett-Kleiner micro colorimeter, with a 1 per cent potassium bichromate solution in a test-tube wedge mounted on a deep yellow glass background. The reading is made and the percentage of urea found by consulting a table.

ANALYSIS AND COMPOSITION OF CORN POLLEN.

By R. J. ANDERSON and W. L. KULP.

(From the Biochemical Laboratory, New York Agricultural Experiment Station, Geneva.)

Having failed to find any reference in the extensive literature dealing with corn and corn products regarding the composition of corn pollen, we have undertaken an investigation of this substance. Since pollen plays an all important part in the process of fertilization and reproduction, it would seem as if some knowledge regarding the chemical compounds occurring in pollen would be of interest to plant physiologists.

Our results indicate that different varieties of corn produce pollen which varies greatly in composition and this fact might be of importance in cross-breeding.

The approximate composition of the pollen from three varieties of corn has been determined and a complete analysis of the ash of the pollen from one variety of corn has been made. Evidence is presented which indicates the presence of at least two phosphatides in corn pollen—one was an amorphous substance which also contained sulfur and the other was crystalline, corresponding in composition to a diaminomonophosphatide. Relatively large quantities of free inosite, *l*-proline, and choline were also isolated.

A COMPARATIVE STUDY OF THE COMPOSITION OF THE FEMUR.

By SERGIUS MORGULIS.

(From the Department of Biochemistry, College of Medicine, University of Nebraska, Omaha.)

A few years ago, through the courtesy of Dr. S. J. Foote, I came into possession of an interesting collection of femurs. In view of the limited number of comparative determinations of bone composition, this material was examined chemically. Without entering here into a discussion of the analytical methods, it may be stated that the bones were cautiously freed from all traces of adhering tissue, dried, and ether extracted. The analyses were, therefore, made on water-free and fat-free material. The present report does not include the data for femurs of Egyptian Mummy or Pueblo Indian. The analyses extended to a determination of organic substance, ash, carbon dioxide, calcium, magnesium, and phosphorus. The quantitative data were calculated as calcium carbonate, magnesium phosphate, and calcium phosphate. Recognizing certain objections to this method of expressing the composition of the bone, it is, however, preferable to the conventional way of giving it in terms of percentages of Ca, Mg, P, and CO_2.

The composition of the bones examined reveals a remarkable uniformity. The percentage of CO_2 in the ash varies from 4.33 to 5.68, that of the Ca from 37.58 to 38.70, of Mg from 0.57 to 0.86, and that of P from 18.03 to 18.90 per cent. The femur of the turtle is an exception in that it contains a much greater proportion of CO_2 (8.43 per cent) and a lower percentage of phosphorus (17.02 per cent) than the other bones studied. Incidentally, it is also the softest bone with the largest content of organic matter.

Hoppe-Seyler regarded the salts of bone tissue as being similar to the mineral apatite $Ca_{10}\ Fl_2\ (PO_4)_6$ in which CO_3 takes the place of Fl_2. In a salt of this composition the Ca as phosphate and as carbonate should be in a ratio of 9.3:1 (930.87:100.08). The last column of the table gives these ratios as determined from our analytical data; the ratio will be seen to vary greatly, from 3.62 in the case of the turtle to 9.11 in the human femur. The ratios for the other femurs examined range all the way between these extremes. The lack of constancy in the ratio between the calcium phosphate and calcium carbonate seems to invalidate Hoppe-Seyler's assumption.

Femur from:	Composition of bone.				Composition of ash			
	Organic matter.	$CaCO_3$	$Mg_3(PO_4)_2$	$Ca_3(PO_4)_2$	$CaCO_3$	$Mg_3(PO_4)_2$	$Ca_3(PO_4)_2$	$\frac{Ca_3(PO_4)_2}{CaCO_3}$
Elk	29.40	7.20	1.59	62.50	10.19	2.55	88.50	8.68
Sheep	29.56	7.76	2.18	60.40	10.92	3.10	85.60	7.84
Mule	30.15	8.03	2.07	61.05	11.49	2.96	87.30	7.60
Hippopotamus	30.26	8.09	1.86	60.90	11.60	2.66	87.40	7.09
Turkey	30.51	7.48	1.86	61.45	10.76	2.67	88.30	8.42
Frog	32.36	8.45	1.66	58.35	12.50	2.45	86.30	6.89
Dog (bull)	32.98	7.42	1.58	57.50	11.08	2.35	85.80	7.98
Man	33.19	6.59	1.40	60.00	9.86	2.09	89.70	9.11
Horse	33.82	8.56	1.36	52.00	12.93	2.06	86.10	6.66
Turtle	37.23	12.03	1.95	49.85	19.16	3.10	79.40	3.62

A STUDY OF THE NON-PROTEIN CONSTITUENTS IN BLOOD OF SOME MARINE INVERTEBRATES.

By SERGIUS MORGULIS.

(From the Department of Biochemistry, College of Medicine, University of Nebraska, Omaha.)

The non-protein components of blood have proved themselves an invaluable aid in the investigation of metabolism of the higher animals. This consideration led the author to undertake a survey of the blood of invertebrates. The present investigation is confined to arthropods from which sufficient blood for analysis can easily be obtained.[12]

The splendid system of blood analysis outlined by Folin and Wu has been followed in this research with some minor modifications necessitated by the nature of the material. This will be fully discussed in the detailed report. The blood from *Limulus*, blue crab, spider crab, and lobster was examined and the results tabulated below.

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[12] It is a pleasure to acknowledge the help received from Dr. Leo Loeb in procuring the bloods.

Non-Protein Constituents of the Blood of Arthropods.

Animal.	Mg. per 100 cc. of blood.			Remarks.
	Sugar.	Non-protein nitrogen.	Uric acid.	
Lobster (*Homarus*).	26	13.0	2.0	
	20	12.8	2.5	
	19	12.5	2.1	
	20	13.3	2.4	
Spider crab (Labinia).	45	33.0	1.0	Fresh batch.
	25	22.0	2.3	“ “
	27	23.0	2.3	“ “
	29	19.5	2.3	“ “
	26	16.0	3.8	?
	43	13.0	2.0	Several days old.
	43	15.0	1.0	“ “ “
Blue crab (Callinectes).	182	24.7	2.9	Fresh from the sea.
	64.5	22.7	3.4	“ “ “ “
	41.5	8.0	0.3	Used on the day of delivery to laboratory.
	17.5	13.6	0.4	
	9.5	9.3	Negative.	1 day in aquarium.
	18.0	18.6	“	
	12.5	9.0	“	2 days in aquarium.
	13.8	10.0	“	
Limulus.	34.0	26.0	0.3	Recently caught.
	22.5	20.0		1 day in tank.
	12.5	14.0	0.7	2 days in tank.
	5.0	10.0		After many weeks in confinement.
	7.5	13.0	0.8	

The findings may be summed up as follows: the sugar, non-protein nitrogen, and uric acid content of the blood shows very great variability, except in the lobsters. The greatest variability occurs in bloods from blue crabs, the smallest in that of the lobster. The uric acid is present in large amount in both lobster and spider crab blood, in blue crabs only in specimens examined almost as soon as they have been removed from the water. The content of the non-protein materials of the blood evidently depends on the condition of the animals (nutritive?). This is especially well shown in the blue crab which is an exceedingly active animal. Examined immediately upon being brought to the laboratory they showed a variable but high content of the non-protein substances, but after having been kept in the aquarium for 1 day the sugar and non-protein nitrogen diminish to practically the lowest level, while the uric acid has disappeared completely. The condition in *Limulus* is similar, and in the spider crab the same state of affairs exists though perhaps not quite so definitely expressed. On the contrary, the blood from lobsters shows very distinctly a stable composition of the non-protein components. The results suggest that in these arthropods different stages in the development of the mechanism for the regulation of the blood composition are represented. The influence of the nutritive state upon the blood composition, and its relation to the degree of development of the excretory mechanism will be the subject of direct experimentation.

THE REACTIVITY OF THE MOLYBDENUM AND TUNGSTEN REAGENTS OF FOLIN.

By VICTOR E. LEVINE and BERNARD C. BURNS.

(*From the Biochemical Laboratory, School of Medicine, Creighton University, Omaha.*)

To test out the reactivity of the Folin reagents 225 compounds of biochemical interest were employed. About 100 compounds responded to the phenol reagent of Folin and Denis. Among these were amino-acids, proteins, aldehydes, ketones, carbohydrates, and alkaloids. Creatinine was positive; creatine, negative. Most compounds reacted at room temperature while others required heating (formic acid, glucose, caffeine, etc.). In view of these findings the phenol reagent is worthless in the detection of the antineuritic vitamine, and the quantitative methods for phenol, cresol, and tyrosine must be modified so as to remove interfering substances from the compound under estimation. This criticism also applies to the estimation of adrenalin with the uric acid reagent.

The introduction of nitro groups in phenol renders the reagent inactive. Mono-, *o*-, and dinitro- and trinitrophenol (picric acid) do not respond. Picraminic acid, however, is positive.

The uric acid reagent proved less reactive, only 45 compounds yielding positive results. Creatinine is positive; and creatine, negative. The estimation of uric acid does not suffer in accuracy from the non-specificity of the reagent, since in the method the uric acid is separated by precipitation from interfering substances.

In the system of blood analysis of Folin and Wu[13] there is recommended in the determination of sugar a reagent reacting with cuprous oxide. It is interesting to find that it reacts with inorganic reducers (cuprous oxide or chloride, ferrous sulfate, sulfites, sulfides, stannous chloride, etc.) and with only two organic compounds, which contain the hydrazine grouping—hydrazine and phenylhydrazine.

THE ACID-BASE EQUILIBRIUM IN THE BLOOD AFTER PARATHYROIDECTOMY.

By D. WRIGHT WILSON and C. I. KRANTZ.

A METHOD FOR THE DETERMINATION OF URIC ACID.

By STANLEY R. BENEDICT.[14]

SOME PHYSIOLOGICAL AND CHEMICAL PROPERTIES OF THYROXIN.

By E. C. KENDALL.

SOME VARIATIONS NOTED IN THE ASH OF THE SWEAT.

By G. A. TALBERT.

NORMAL SUGAR EXCRETION IN RELATION TO CARBOHYDRATE INTAKE AND BLOOD SUGAR FLUCTUATIONS.[15]

By OTTO FOLIN and HILDING BERGLUND.

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[13] Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, xli, 367.

[14] Benedict, S. R., *J. Biol. Chem.*, 1922, li, in press.

[15] Folin, O., and Berglund, H., *J. Biol. Chem.*, 1922, li, in press.

PROTEIN METABOLISM FROM THE STANDPOINT OF BLOOD AND URINE ANALYSIS.

By W. S. McELLROY and H. O. POLLOCK.

A RAPID METHOD FOR THE DETERMINATION OF GLYCOGEN IN TISSUES.

By ELMER L. SEVERINGHAUS.

THE KETOLYTIC ACTION OF GLUCOSE IN VITRO.

By P. A. SHAFFER and T. E. FRIEDMANN.

A NEUTRAL GASTRIC ANTACID WHICH DOES NOT ACT AS A SYSTEMIC ALKALI.

By ISIDOR GREENWALD.

A STUDY OF THE BASAL METABOLISM, THE SUGAR TOLERANCE, AND THE GOETSCH TEST IN CASES OF THYROIDISM.

By JOSEPH S. HEPBURN and HARRY M. EBERHARD.

ABSTRACT OF THE EXECUTIVE PROCEEDINGS OF THE SIXTEENTH ANNUAL MEETING.

NEW MEMBERS: Katharine Blunt, Mary Van R. Buell, Casimir Funk, A. B. Hastings, J. A. Killian, C. A. Mills, A. H. Smith.

RESIGNATION ACCEPTED: Oskar Klotz.

DECEASED MEMBERS: A. C. Crawford, Gustav Mann.

OFFICERS FOR THE YEAR 1922: *President*, Donald D. Van Slyke; *Vice-President*, Philip A. Shaffer; *Secretary*, Victor C. Myers; *Treasurer*, Walter R. Bloor; *Additional Members of the Council*, Stanley R. Benedict, Harold C. Bradley, Albert P. Mathews. *Nominating Committee*, C. L. Alsberg, G. H. A. Clowes, P. B. Hawk, H. B. Lewis, P. A. Levene, E. V. McCollum, L. B. Mendel, J. R. Murlin, R. T. Woodyatt.

EDITORIAL COMMITTEE, elected to serve 1922–27: T. B. Osborne, W. W. Palmer, A. N. Richards.

DUES: The dues for the year 1922 were fixed at $2.00. Eighty-nine members of the Society attended the meeting.

VICTOR C. MYERS,
Secretary.

PROCEEDINGS

OF THE

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

SEVENTEENTH ANNUAL MEETING

Toronto, Canada

December 27–29, 1922

ABSTRACTS OF PAPERS
ABSTRACT OF EXECUTIVE PROCEEDINGS

Volume VI
No. 1

PROCEEDINGS OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS.

SEVENTEENTH ANNUAL MEETING.

Toronto, Canada, December 27–29, 1922.

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Abstract of Executive Proceedings........................ 48

For the List of Members and the Constitution and the By-Laws of the Society see the 1923 Register of the Federation of American Societies for Experimental Biology, comprising the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, and the American Society for Experimental Pathology.

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THE EFFECT OF ANOXEMIA ON METABOLISM.

BY E. J. SCHNELLER, E. H. BRUNQUIST, AND A. S. LOEVENHART.

(*From the Pharmacological Laboratory of the University of Wisconsin, Madison.*)

It has been found in our laboratory that a reduction of oxygen in the respired air to from 6 to 9 per cent results, first, in a condition of alkalosis, as has been previously found by Haggard and Henderson, and later in the production of a very marked acidosis. The extent of the acidosis depends on how much we lower the oxygen content of the atmosphere which the animal breathes.

The relation of anoxemia to acidosis is being presented elsewhere but it is necessary to give this brief résumé in order to interpret the changes in the metabolism.

Anoxemia steadily maintained from 3 to 5 days, causes: (1) an increase in the endogenous nitrogen metabolism. (2) Induces an increase in the excretion of incompletely degraded metabolites, as shown by the increased elimination of total organic acids. (3) Changes the creatine and creatinine ratio, the creatine excretion being much increased and becoming greater in many cases than the creatinine excretion.

THE URINARY SULFUR OF FASTING STEERS.

BY THORNE M. CARPENTER.

(*From the Nutrition Laboratory, Carnegie Institution, Boston.*)

Two steers of about 600 kilos body weight were fasted for 5, 7, 10, and 14 days. The urines were collected every 24 hours. The inorganic sulfate, total sulfates, and total sulfur were determined in these urines by Fiske's[1] method. The maximum and minimum values for the three forms of sulfur (calculated as sulfur) were as follows: inorganic sulfate, 2.88 and 0.02 gm.; the ethereal sulfate, 4.23 and 0.05 gm.; neutral sulfur, 1.73 and 0.11 gm.

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[1] Fiske, C. H.. *J. Biol. Chem.*, 1921, xlvii, 59.

The percentage distribution ranged as follows: inorganic sulfate, 0.4 to 75.6 per cent; ethereal sulfate, 2.7 to 93.7 per cent; and neutral sulfur, 8 to 50 per cent. In general, however, the percentage of neutral sulfur was more constant than the other two. The nitrogen sulfur ratio was fairly constant in all the fasts except in the 7 day fast. Its value averaged about 20:1.

FURTHER OBSERVATIONS ON THE CHEMICAL COMPOSITION OF THE BODY FLUIDS OF THE SEA-LION.

By ROBERT E. SWAIN and N. W. RAKESTRAW.

(*From the Department of Chemistry, Stanford University, Stanford University.*)

Analyses were made of the blood, pericardial fluid, milk, and urine of ten sea-lions (*Eumetopias stelleri*). The samples were taken from animals killed on the rookery on Ano Nuevo Island, off-shore from Pescadero, California. A few especially noteworthy results are the high fat content of the milk (24.8 per cent), and total solids amounting to 50 per cent. In the blood unusually high values, with wide variations, were found in total N, non-protein N, amino-acid N, and urea. A peculiar condition arose in connection with uric acid. None was found in the blood or pericardial fluid by the method of Folin-Wu, but good values were indicated by the methods of Benedict and Morris and Macleod, although these showed poor agreement, but a constant variation. A remarkable increase in the uric acid in the blood, but not in the pericardial fluid, was apparent in several cases on standing. Uric acid was found in the urine. Extraordinarily high values were obtained for total lipoids in the blood and pericardial fluid.

SYNTHESIS OF ORNITHINE IN THE FOWL.

By JAMES H. CROWDLE and CARL P. SHERWIN.

(*From the Chemical Research Laboratory, Fordham University, New York.*)

Previous work has shown that the animal placed on a carbohydrate diet whereby the nitrogen metabolism was reduced to a minimum, is still able to synthesize two of the amino-acids (glycocoll and glutamine) from endogenous waste nitrogen, which would otherwise appear as urea nitrogen. Cystine cannot, however, be synthesized under these circumstances.

In order to determine the possibility of ornithine synthesis, a hen was provided with prenatural anus which allowed the separate collection of urine and feces. The hen was kept on a non-nitrogenous diet until the endogenous urinary nitrogen excretion was constant. She was then fed benzoic acid. Both free and combined benzoic acid were determined. The combined benzoic acid was identified as the dibenzoyl ornithine. Total nitrogen, urea nitrogen, uric acid nitrogen, and ammonia nitrogen were determined.

The feeding of the acid caused a slight rise in total nitrogen, a relative decrease in uric acid nitrogen, but a decided increase in the other nitrogenous urinary excretions, which seems to indicate the synthesis of ornithine at the expense of the uric acid nitrogen. Finally, along with the benzoic acid were fed first, proline, then histidine, and lastly arginine. The latter added substantially to the output of ornithine. Proline in a less degree increased the ornithine synthesis, but histidine seemed to have little effect. All these amino-acids were readily catabolized and a greater part of their nitrogen was excreted in the uric acid fraction or ammonia fraction.

THE EFFECT OF CERTAIN NITROGEN COMPOUNDS UPON THE ACTIVITY OF UREASE.

By E. W. ROCKWOOD and W. J. HUSA.

(From the Laboratory of Physiological Chemistry, University of Iowa, Iowa City.)

Jack bean urease was used. The nitrogen compounds were used in 0.001 M concentration. The pH was kept at 7.5 by 0.5 M phosphate buffer solution. Fermentation continued about 2 hours at room temperature, then K_2CO_3 was added and the ammonia removed by aeration. The α-aminocarboxylic acids markedly accelerated the fermentation, the β acids much less. The greater the number of carbon atoms between the NH_2 and CO_2H group the less was the effect; increasing the length of the carbon chain also decreased the promoter effect. Amines and acid amides had no accelerating action, nor did creatine, creatinine, or uric acid. Stereoisomeric amino-acids showed no definite difference. The carboxyl group alone or the amino group alone is inactive.

THE METABOLISM OF GALACTOSE.

I. THE THRESHOLD OF TOLERANCE IN NORMAL INDIVIDUALS.

By ALLAN WINTER ROWE.

(From the Department of Chemistry, Evans Memorial Hospital, Boston.)

Provocative galactosuria was suggested in 1906 by Bauer as a test for liver function. The fairly extensive literature is chiefly clinical in character. The "carbohydrate anomaly" is ignored and positive reactions are recorded only on appearance of relatively large amounts of sugar. With the complex control mechanism of carbohydrate assimilation and utilization, the test is susceptible of other application and possible diagnostic use suggested. Since it is necessary to establish the normal threshold, a series of studies has been made on the normal man and woman using galactose of highest purity and carrying out, on standard diet, 7 day studies of general metabolism and functional capacity with the sugar tests as the final step.

	17 women.		16 men.	
	Range.	Average.	Range.	Average.
Age, *yrs*	19–52	26	20–70	29
Weight, *kg*	47.0–89.1	58.8	54.8–83.2	67.8
Height, *cm*	151–181	162	164–182	172
Area, *sq. m*	1.44–1.99	1.61	1.54–1.98	1.80

The urines were tested qualitatively by Benedict's solution and on appearance of sugar, amounts were quantified.

	gm.
Average exciting dose in men	27.4
" " " " women	38.5

It is suggested that the evident sex difference rests on Folin's tissue absorption theory and potential mammary function in females. A series of blood sugar curves tend to show the character of the curve conditioned by the size of the dose. Of six anomalous curves, five were with doses below that exciting a melituria. Numerous other curves show normal rise and fall.

In pathological cases showing high threshold, 15 gm. of urea were given with the galactose test meal. Blood and urine urea curves show that the high level was not dependent upon retarded absorption. It is concluded that the dose of galactose exciting a definite melituria is of the order of 30 gm. for men and 40 gm. for women.

POSTMORTEM ACIDITY.

By ELMER L. SEVRINGHAUS.

(*From the Laboratory of Physiological Chemistry, University of Wisconsin, Madison.*)

Autolyzing liver undergoes small changes in hydrogen ion concentration. If alkali is added to the autolyzing tissue the initial hydrogen ion concentration approaches neutrality very rapidly at first, then with a decreasing rate. This same return to neutrality is observed with acidified tissues, but the changes are not so large, and the tissues remain definitely acid. Both bases and acids must be formed during autolysis. The amphoteric amino-acids do not seem adequate to account for this buffering. A comparison of the total water-soluble acidity in such autolyzing livers, by titration with phenolphthalein, shows that the acidified tissues develop more acid than the control or the alkaline ones. These acids must be weak acids, because they appear with the hydrogen ion concentration changing rather little in the acid tissues, but markedly in the alkaline tissues. The water-soluble acids are sufficient in amount to account for 60 to 80 per cent of the buffering of the alkali. Fatty acids also play a small part, carbon dioxide less, and lactic acid probably still less.

Phosphoric acid, determined by the Bell-Doisy and the Briggs methods as inorganic phosphate, appears to make up most of the water-soluble acidity. The inorganic phosphate content of dog's liver is 15 to 20 mg. per 100 gm. of liver, in samples taken 7 to 20 minutes after the death of the animal. This amount increases rapidly at first, then more slowly to a total of 250 to 300 mg. per 100 gm. of liver after 20 days. One-third or more of the total inorganic phosphate is liberated by the end of 6 hours after death. The addition of alkali increases it. These effects are proportional to the amounts of alkali or acid.

The acidified tissues develop more total acid, but less phosphoric acid than the others, and therefore this must be due to some other weak acid. It is suggested that phosphoric acid may be a specific buffer mechanism *versus* alkali development in tissues.

HYDROGEN ION CHANGES ACCOMPANYING DEATH.

By ALFRED E. KOEHLER.

(From the Laboratory of Physiological Chemistry, University of Wisconsin, Madison.)

In the study of the reaction of autolyzing liver brei it was noted that the initial pH several hours after death was in the neighborhood of 6.6. In greatly shortening the period after death it was found that the increase in acidity is not a gradual one but that a reaction of about pH 6.7 is developed in the first minutes which does not change subsequently to any marked extent. In this study dogs were killed practically instantaneously by shooting and the tissues removed at once, ground to a brei, and mixed with enough water or 0.8 per cent NaCl solution so as to flow into a hydrogen electrode vessel.

Similar results were obtained with muscle tissue. In an attempt to eliminate the macerating effect upon the tissue, perfusing the leg of a dog with adjusted Ringer's solution was tried. Death resulted in a rapid and marked change of the perfusate from a pH of 7.3 before to 6.8 after death.

THE RISE OF ACIDITY IN AUTOLYSIS.

By WITHROW MORSE.

(From the Department of Physiological Chemistry, School of Medicine, West Virginia University, Morgantown.)

Studies upon different organs of guinea pigs and of rabbits show a depletion of the alkali reserve of these tissues immediately after death. The mercury pump of Van Slyke was used in the determinations, the method being followed as used for blood plasma. The organs were excised after rapid laparotomy following the killing of the animal by occipital stroke. The heart was beating during excision. The organs were placed under oil, ground in sand, centrifuged, and the layer beneath the oil was used for the determinations. The tissues (kidney, spleen, and liver) showed a degree of carbon dioxide-combining power below that exhibited by the blood plasma of a patient in diabetic coma.

THE PRODUCTION OF ACIDOSIS BY ANOXEMIA.

By A. E. KOEHLER, E. H. BRUNQUIST, and A. S. LOEVENHART.

(From the Departments of Pharmacology and Physiological Chemistry, University of Wisconsin, Madison.)

The view that decreased oxidation in the body leads to the accumulation of acid products has been held for many years, and it has often been assumed that decreased oxidation leads to acidosis. Yandell Henderson and Haggard questioned this conclusion and demonstrated a decrease in the H ion concentration of the blood, *i.e.* alkalosis, as a result of short periods of decreased oxygen supply. They questioned whether decreased oxidation can produce acidosis. The alkalosis found by Henderson is due to the rapid excretion of CO_2 by the lungs. It was the view of the writers that decreased oxidation does lead to acidosis and their views were put to test by the following method.

Pigs were exposed to atmospheres low in oxygen with a low CO_2 content. The respiratory chamber, described by Dallwig, Kolls, and Loevenhart, was used. The electrometric determination of the pH of the blood was made together with the total CO_2. The authors confirmed the findings of Henderson and Haggard and found a very definite increase in the pH of the blood from 7.4 to the region of 7.55 in the early stages of anoxemia. On continuing the anoxemia several hours, the pH returns to normal and continues to decrease. Marked increase in the respiratory rate begins as soon as the animals are exposed to low oxygen and continues throughout the experiment until near the end. The respiratory rate rises from the normal of approximately 40 to 200 per minute. There is but a short interval of time between the fall in the respiratory rate and death. The decrease in the respiratory rate indicates exhaustion of the respiratory center. At this time, we have frequently found that the pH of the blood slows to 6.7 several minutes after the animal is removed from the chamber into the ordinary atmosphere. If the animal recovers, the return to normal is rapid—usually being complete in less than an hour—both in regard to blood pH and CO_2 content. The pH may then rise above normal (7.55). This alkalosis may last 1 hour, but the normal is finally reached. At the height of acidosis, the total CO_2 of the blood may fall to 10 per cent. The rise in CO_2 proceeds simultaneously with the rise in the blood pH.

The data prove the development of acidosis as a result of decreased oxygen supply. We believe that the production of fixed acids begins at once but does not keep pace with the loss of CO_2 from the blood, so that alkalosis is first noted. Later, the acid production more than compensates for the loss of CO_2 with the resulting very low pH values. Various phases of this work are being continued with the cooperation of Messrs. H. M. F. Behneman, O. E. Benell, and H. L. Schmitz.

THE REFRACTIVE INDEX AND WATER CONTENT OF THE BLOOD SERUM OF THYROPARATHYROIDECTOMIZED AND PARATHYROIDECTOMIZED ALBINO RATS.

By FREDERICK S. HAMMETT.

(*From The Wistar Institute of Anatomy and Biology, Philadelphia.*)

Albino rats were thyroparathyroidectomized or parathyroidectomized at 100 days of age. Determinations were made of the refractive index and water percentage of the blood serum at 150 days and compared with that of their litter controls.

It was found that thyroparathyroidectomy induced an absolute increase in the refractive index and a decrease in the water content of the serum of the male rats. The serum of the female thyroparathyroidectomized rats did not differ from that of the controls in absolute values. Parathyroidectomy did not produce alterations in the absolute values of the serum of either the males or females.

The refractive index of the serum of albino rats is a linear function of the water percentage. It is thus possible to determine whether or not a given experimental procedure has caused a change in the chemical nature of the substances (aside from water) giving rise to refraction by equating the observations for any given set of groups and comparing the slopes and the positions of the lines so obtained. The results of such an analysis show that: (1) the chemical nature of the serum of normal female rats is the same as that of the normal males; (2) thyroparathyroidectomy of male rats does not induce alterations in the chemical nature of the refracting substances. Hence the decrease in water content of the blood serum of the rats of this group is a real desiccation without chemical change. (3) Parathyroidectomy of both male and female rats causes a marked and significant change in the nature of the refractive substances. This change is in the same direction and of the same order of magnitude for both sexes. The comparison of the effect produced by the loss of the parathyroids with that produced by the loss of the thyroid apparatus clearly shows a fundamental dissimilarity in the respective metabolic upsets. (4) Thyroparathyroidectomy of the females induced an alteration in the nature of the refractive substances of the serum in the same direction as that produced by parathyroidectomy. The difference in response of the females from that of the males which were thyroparathyroidectomized accompanied a much more marked growth retardation in the former. In fact, the female thyroparathyroidectomized rats lost weight immediately after the removal of the glands and never regained their initial status. This fact is sufficient to account for the change in the nature of the refractive substances in this group as compared with the males of the same group and the controls.

THE RELATION OF FRACTURE HEALING TO THE INORGANIC PHOSPHORUS OF THE BLOOD SERUM.

By WALTER H. EDDY and HATTIE L. HEFT.

(From the Department of Pathology, New York Hospital, and the Department of Physiological Chemistry, Teachers College, Columbia University, New York.)

Tisdall and Harris have recently presented data[2] which suggest a direct relation between the inorganic phosphorus content of the blood serum and the progress of fracture healing. Their article stimulated the series of studies which we have begun with the cooperation of Drs. C. R. Murray and Ralph Stillman of the New York Hospital. In this preliminary report we wish to present some of our findings to date.

The blood of twenty-three fracture cases has been examined to date, several of them repeatedly. Practically all these cases show an abnormally high phosphorus and the progress of healing bears a definite relation to the amount of phosphorus found. One non-union case which showed a normal amount 40 days after fracture promptly developed a rise following operative reduction and the use of a caliper splint. These findings confirm those of Tisdall and Harris.

We have also, however, examined the bloods of some twenty cases involving surgical operation and whose recovery involved the healing of tissue other than bone. The same high phosphorus is found in these cases also. CO_2 capacity determinations were made on all the cases examined and the results cannot be laid to variations in this factor. This second result suggests that we must at least proceed cautiously before accepting the phosphorus findings as a specific index of bone formation and the desirability of more detailed inquiry into what makes the increase.

Dr. Murray suggested that it was a common belief that syphilis is an inhibiting influence in fracture healing. Examination of the bloods of some thirty positive Wassermann cases fails to show abnormal phosphorus findings in these cases. In regard to the normal value, however, we take exception to Tisdall and Harris' curve. Our studies of normal bloods of persons between the ages of 15 and 65 indicate that 2.9 mg. of phosphorus per 100 cc. of serum is nearer the normal figure.

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[2] Tisdall, F. F., and Harris, R. I., *J. Am. Med. Assn.*, 1922, lxxix, 884.

Finally, we wish to add that the above determinations have been made by the Tisdall method. We have checked this method repeatedly against the Briggs modification of the Bell-Doisy method and find that the two methods give equally accurate results, but we find the greens of the Tisdall method present less difficulty in matching colors.

THE PHOSPHORUS AND CALCIUM CONCENTRATION OF THE SERUM OF PATIENTS DURING THE PERIOD OF FRACTURE UNION.

By J. J. MOORHEAD, H. W. SCHMITZ,* LOIS CUTTER, and V. C. MYERS.

(From the Departments of Traumatic Surgery and Biochemistry, New York Post-Graduate Medical School and Hospital, New York.)

In 1920 a few calcium estimations were made on fracture cases with non-union, also cases with very rapid bone regeneration, but these analyses did not appear to disclose any abnormality in the calcium content of the serum.[3] Our interest in this problem has again been aroused by the important observation of Tisdall and Harris,[4] that during the period of union of fractures in adults, the phosphate content of the serum is raised to a level approximately the same as that present in childhood. The present preliminary study includes observations on about ninety cases, thirty of which were fracture cases. The method of Briggs[5] was employed for the inorganic phosphorus and that of Clark[6] for the calcium.

The average inorganic phosphorus found in thirty normal adults was 3.7 mg. per 100 cc., while in ten children under 10 years it was slightly below 5.0 mg. The low average observed in children is probably to be explained by the fact that some of the cases were undernourished.

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* Medical Fellow of the National Research Council.

[3] Myers, V. C., Chapter on body tissues and fluids, in Barker, L. F., Endocrinology and metabolism, New York, 1922, iii, 451.

[4] Tisdall, F. F., and Harris, R. I., *J. Am. Med. Assn.*, 1922, lxxix, 884.

[5] Briggs, A. P., *J. Biol. Chem.*, 1922, liii, 13.

[6] Clark, G. W., *J. Biol. Chem.*, 1921, xlix, 487.

The inorganic phosphorus of the blood serum has generally been found to rise following fracture, but in our series 5.0 mg. or more have been attained in rather less than half the cases. The highest values are sometimes observed in 2 or 3 days after the fracture, at other times not until a month has elapsed. In one case union was observed, although there was only a slight rise in the phosphorus, 2.5 to 3.2 mg. In another case there was non-union, although the phosphorus reached 5.0 mg. after 3 days and did not drop to 3.8 until a month later. In a series of six fracture cases in children phosphorus values of 5 mg. were observed in only one case, although in the four cases having more than one observation all showed a rise. In two cases with mal- and non-union there was a rise in the phosphorus following operation. In two cases of non-union, however, there was a slight drop following operation. One of these still shows non-union at the end of 2 months. A series of nine cases with minor fractures (fingers) discloses negative results. Several of the cases appear to show slight variations in the calcium, paralleling the phosphorus.

OBSERVATIONS ON THE NATURE OF THE CARBOHYDRATE OF NORMAL URINE.

By ISIDOR GREENWALD.

(*From the Harriman Research Laboratory, Roosevelt Hospital, New York.*)

The excretion of sugar in the urine of a man and of a dog was as high on a protein-fat diet furnishing an adequate amount of energy as it was on a carbohydrate diet. The 24 hour quantity was not increased in the dog, by the ingestion of as much as 10 gm. of glucose per kilo, when the animal was on a carbohydrate diet. When the same quantity was given with a protein-fat diet, there was a slight increase in the quantity of sugar in the urine, the increase being the greater, the larger the amount of fat in the diet. The man showed an apparently perfect tolerance for 1 gm. of glucose per kilo body weight when on a diet containing about 50 gm. of fat and furnishing about 11 gm. of nitrogen in the urine. When the fat was increased to about 140 gm. per day, there was a slight increase in the amount of sugar in the urine. On the protein-fat diet, about 0.05 gm. of glucose per kilo body weight appeared in the urine. The excretion of sugar was not affected by the ingestion of 12 gm. of thymus, or yeast nucleic acid, by the man.

Several osazones were obtained from mixed normal human urine. One of these was identified as lactosazone. Another is probably pentosazone, for pentose was demonstrated in other ways to constitute about one-third of the total sugar. An osazone insoluble in hot water and melting at about 200° was also obtained, but under the microscope appeared to be a mixture.

It is suggested that pentose may be formed from protein and that pentosuria is a disorder of protein, not of carbohydrate, metabolism.

A STUDY OF THE NON-CYSTINE-PROTEIN SULFUR.

By J. HOWARD MUELLER.

(From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York, and the Biochemical Laboratory, Cambridge University, England.)

Some time ago the writer reported at the Society of Experimental Biology and Medicine in New York, the isolation of crystalline material from a sulfuric acid hydrolysate of casein. After repeated recrystallization, this material had the composition represented by the formula $C_{11}H_{23}SN_2O_4$. Further work has shown that it contained considerable phenylalanine and at least one other unidentified amino-acid in addition to the sulfur compound. It has now been possible by two quite different methods to isolate the sulfur compound in a much purer form, corresponding quite closely to the formula $C_5H_{11}SNO_2$. It has been obtained after both sulfuric acid and sodium hydroxide hydrolysis of casein in about the same yield, and also from several other proteins. The sulfur is not in the lead blackening form, and does not give a red color with sodium nitroprusside in the form isolated, nor after reduction. The nitrogen is split off quantitatively by nitrous acid, indicating an α-NH_2 group; CO_2 is given off on heating and salts are formed with metals, and a COOH group, therefore, appears to be present.

Fusion with NaOH, and heating in a sealed tube produce a volatile sulfur compound giving a red color with sodium nitroprusside. The structure is being further investigated.

THE SEPARATION OF THE HEXONE BASES FROM CERTAIN PROTEIN HYDROLYSATES BY ELECTROLYSIS.

By CARL L. A. SCHMIDT AND G. L. FOSTER.

(From the Department of Biochemistry and Pharmacology, University of California, Berkeley.)

Experiments have been carried out with hydrolyzed gelatin, casein, and fibrin, which show that the hexone bases can be separated from the other amino-acids by electrolysis. The hydrolysate is placed in the center of a three-compartment cell and distilled water is placed in each of the end compartments. These are separated by gelatin membranes and carbon electrodes are used. On passing a current through the cell the hexone bases wander to the cathode. Glutamic and aspartic acid and the pigment migrate to the anode, while the other amino-acids remain in the center compartment. When the pH of the center compartment is kept at 7.5 histidine does not wander to the cathode but at pH 5.5 the three hexone bases migrate to the cathode in about the same ratio as they existed in the protein.

IODINE IN NATURAL WATERS IN RELATION TO GOITER.

By J. F. McCLENDON.

(From the Laboratory of Physiological Chemistry, University of Minnesota Medical School, Minneapolis.)

Kendall found that foodstuffs lose iodine in charring, but I burn them in a calorimeter-bomb to prevent loss. Foodstuffs derive iodine from soils, and river water represents leachings from soils. In the Mississippi river system, in Minnesota, iodine averages 0.8 (per billion) and goiter 8 (per 1,000 troops); in Missouri, iodine is increased to 2 and goiter reduced to 4.

The distribution of Cl and I might show similarity because of similar solubility. The United States is divided into four regions: first, goiter 15 to 30 (per 1,000 troops) and Cl 0.5 to 2 (per cent of total dissolved solids in representative rivers); second, goiter 5 to 15 and Cl 1 to 3; third, goiter 1 to 5 and Cl 2 to 5; fourth, goiter 0 to 1 and Cl 4 to 30.

In the iodine determination, 100 liters of water are made alkaline, evaporated to dryness, and charred in the bomb with threads, water-cooled to prevent welding. The char is leached with boiling water and iodate and iodide changed to iodine and determined in CCl_4 with a Bausch and Lomb colorimeter with a cup holding 1 cc.

Rats fed KI by myself for two generations had thyroids averaging half the weight of those of Dr. C. M. Jackson's rats fed no iodine.

THE EFFECT OF SOME ORGANIC ACIDS ON THE EXCRETION OF URIC ACID BY MAN.

By HARRY V. GIBSON and EDWARD A. DOISY.

(*From the Laboratory of Biological Chemistry, Washington University School of Medicine, St. Louis.*)

In an attempt to analyze the effect of amino-acids upon the excretion of uric acid[7] possible deamination products were ingested by two normal men. Pyruvic acid produces a rise while lactic acid causes a very sharp decrease in the hourly excretion of uric acid.

The effect of lactic acid suggests a partial explanation of the curve of excretion of uric acid[8] during and following exercise. The lactic acid produced may well account for the decreased excretion of uric acid during exercise, but *per se* probably does not explain the increased purine elimination.

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[7] Lewis, H. B., Dunn, M. S., and Doisy, E. A., *J. Biol. Chem.*, 1918, xxxvi, 9.

[8] Kennaway, E. L., *J. Physiol.*, 1909, xxxviii, 1.

A CHEMICAL STUDY OF SALIVA.

By J. LUCIEN MORRIS and VERNON JERSEY.

(From the Biochemistry Department of the Western Reserve University School of Medicine, Cleveland.)

Saliva from adults and children was analyzed. The secretion was collected from the quiescent glands during fasting and varied diets. Observation was made of the effect of mechanical and chemical stimuli.

Increase of cellular activity took place as the day progressed. Urea, uric acid, creatinine, and amino-acids in the secretion were readily determined by microchemical methods. Reducing sugar was not present in normal saliva. Urea and amino-acids showed variations proportional to volume changes. Uric acid and creatinine were more constant and relatively more independent of the volume.

EFFECT OF FOOD INGESTION ON THE CLOTTING TIME OF THE BLOOD.

By C. A. MILLS.

(From the Department of Biochemistry, College of Medicine, University of Cincinnati, Cincinnati.)

The clotting time of the blood is longest before breakfast in the morning, but shows only slight variations at this time on successive days. It shortens 30 to 40 per cent about an hour after each meal and 2 to 3 hours later begins to lengthen. 5 or 6 hours after each meal it will be almost at the same point as on rising in the morning. This clotting time observed before each meal is termed the "basal clotting time."

The cause of this effect of food ingestion remains in obscurity. The clotting time bears no relation to the specific gravity of the blood so far as physiological variations go. Water taken produces no change in the clotting rate; neither does glucose solution nor eating plain sugar candy. Coagulated egg white produces no change for about an hour after eating, but later causes the typical increased coagulability of the blood. The effect on the blood then, seems to depend on the presence of the food in the intestine.

Whether it is the absorbed material that causes the change cannot be said. Acid or alkali ingestion sufficient to alter markedly the reaction of the urine produces no change in the clotting time.

THE INFLUENCE OF CARBON DIOXIDE ON THE VELOCITY OF SEGMENTATION OF SEA URCHIN EGGS.

By G. H. A. CLOWES and HOMER A. SMITH.

(*From the Biochemical Research Laboratory, Eli Lilly and Company, Indianapolis.*)

Methods have been devised whereby the influence of CO_2 on the segmentation of sea urchin eggs and larvæ could be studied in a quantitative manner. It has been found that the velocity of segmentation is an *inverse linear function* of the concentration of CO_2. This function is independent of the concentration of hydrogen, sodium, or calcium ions in the sea water (within those limits, of course, which are compatible with normal development), and it is apparently unaltered by variations in temperature between 15 and 30°C. CO_2 causes a progressive retardation, or, at definite tensions, complete arrest of development at all stages from the freshly fertilized egg to the larval stage without causing any alteration in normal cell structure (cytolysis, abnormal division, etc.) such as are caused by most deleterious agents.

A GAS ANALYSIS APPARATUS FOR USE WITH CHAMBER RESPIRATION APPARATUS.

A DEMONSTRATION.

By THORNE M. CARPENTER.

(*From the Nutrition Laboratory, Carnegie Institution, Boston.*)

The gas analysis apparatus is constructed on the Haldane principle. The graduation of the burette is so designed that a carbon dioxide content up to 1.700 per cent and an oxygen deficit up to 2.000 per cent can be determined. From the tap to the first graduation is a bulb with a capacity of 31.36 cc. From 31.36 to 31.84 cc. there is a capillary tube with divisions of 0.004 cc. From 31.84 to 39.36 cc. is a second bulb and from 39.36 to 40.04 cc. there is another capillary tube divided so that each division corresponds to 0.004 cc. The burette is marked in percentage and the smallest division corresponds to 0.01 per cent and estimations may be made to 0.001 per cent. The pyrogallic acid container is made after the pattern of Krogh's apparatus.[9] The following series of analyses of outdoor air and analyses of air from a chamber with an ethyl alcohol flame in it illustrate the results obtainable with the apparatus.

Analyses of outdoor air.		Analyses of air from an ethyl alcohol flame.		
Analyst: E. L. F., Sept. 26, 1922.		Analyst: M. L. B., July 22, 1922.		
CO_2	O_2	CO_2	O_2 deficit.	R. Q.*
per cent	*per cent*	*per cent*	*per cent*	*per cent*
0.027	20.939	0.638	0.970	0.658
0.031	20.942	0.665	1.002	0.664
0.029	20.944	0.664	1.009	0.660
0.034	20.940	Analyst: L. A. R., Oct. 12, 1922.		
0.030	20.939			
		0.551	0.845	0.652
		0.512	0.773	0.662
		0.358	0.532	0.673

* Theoretical R. Q. = 0.667.

THE ESTIMATION OF BILE ACIDS IN ICTERUS URINE.

By CARL L. A. SCHMIDT and JOHN A. MERRILL.

(*From the Department of Biochemistry and Pharmacology, University of California, Berkeley.*)

The procedure is as follows: The urine is evaporated at a low temperature, the bile acids are dissolved in absolute alcohol, the alcohol is evaporated, and the bile acids are taken up in a small quantity of water and precipitated in the cold by saturation with $MgSO_4$. The precipitate is washed with a saturated solution of $MgSO_4$ and then dissolved in absolute alcohol. The alcohol is evaporated and the amino nitrogen is estimated in separate portions of the aqueous solution before and after hydrolysis with 8 per cent NaOH. The difference gives the nitrogen of the bile acids.

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[9] Krogh, A., *Biochem J.*, 1920, xiv, 269.

THE BENZOATE TEST FOR RENAL FUNCTION.

By F. B. KINGSBURY.

(*From the Laboratory of Physiological Chemistry, University of Minnesota Medical School, Minneapolis.*)

44 tests have been made on normal individuals and 39 on patients with renal disorders. A few comparisons have been made with the urea concentration test of MacLean,[10] the water excretion test of Volhard,[11] the constant Austin, Stillman, and Van Slyke,[12] and the phenolsulfonephthalein test of Rowntree and Geraghty.[13] A fairly close agreement has been found between the findings by the benzoate test and by the others with the exception of the phenolsulfonephthalein test in cases of hypertension and the nephrosis due to the toxemia of pregnancy. In well marked nephritis the benzoate test is always low and the findings agree with the blood urea and phenolsulfonephthalein criteria. The benzoate test indicates impairment of renal function earlier than does the retention of urea in the blood.

The benzoate tests were made uniformly as follows: The subject voids urine and immediately thereafter drinks a solution of 2.4 gm. of sodium benzoate in 100 cc. of water. The container is rinsed with about 200 cc. of water and this also is drunk. At the end of 1 hour 200 cc. of water are drunk. 2 hours after ingesting the sodium benzoate the subject collects the first specimen of urine. 1 hour later the second sample of urine is collected. The test is made before breakfast and all food avoided until after the test. The urine samples are separately analyzed for hippuric acid by the method of Kingsbury and Swanson.[14]

The total excretion of hippuric acid in the first 2 hours has been shown normally to be at least 70 per cent of that theoretically obtainable from 2.4 gm. of sodium benzoate and the total for 3 hours to be at least 90 per cent.

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[10] MacLean, H., and de Wesselow, O. L. V., *Brit. J. Exp. Path.*, 1920, i, 53.

[11] Volhard, F., and Fahr, T., Die Brightsche Nierenkrankheit Klinik, Pathologie und Atlas, Berlin, 1914, 121 and 126.

[12] Austin, J. H., Stillman, E., and Van Slyke, D. D., *J. Biol. Chem.*, 1921, xlvi, 91.

[13] Rowntree, L. G., and Geraghty, J. T., *J. Pharmacol. and Exp. Therap.*, 1909–10, i, 579.

[14] Kingsbury, F. B., and Swanson, W. W., *J. Biol. Chem.*, 1921, xlviii, 13.

The findings in this report confirm those of Kingsbury and Swanson[15] and extend the usefulness of this test.

My thanks are due to Dr. George E. Fahr of the Department of Medicine for helpful criticism and for his cooperation in furnishing data of the other renal tests mentioned.

THE SYNTHESIS AND RATE OF ELIMINATION OF HIPPURIC ACID IN THE ORGANISM OF THE RABBIT.

By HOWARD B. LEWIS AND WENDELL H. GRIFFITH.

(*From the Laboratories of Physiological Chemistry of the University of Illinois, Urbana, and of the University of Michigan, Ann Arbor.*)

Previous studies on the precursors of the glycocoll made available for the detoxication of benzoic acid have been unsatisfactory, inasmuch as the rabbit, the experimental animal usually employed, can conjugate almost completely any non-lethal dose of benzoate in a period of 24 hours, regardless of the presence of possible precursors of glycocoll. It seemed probable that in the presence of a metabolite which could form glycocoll the velocity of the formation and elimination of hippuric acid after benzoate ingestion might be increased, and accordingly, studies of the rate of elimination of hippuric acid over short periods of time have been made in rabbits. Hippuric acid administered intravenously was eliminated almost quantitatively in 6 hours, while after intravenous injection of an equivalent quantity of sodium benzoate, less than 50 per cent was eliminated as hippuric acid in a like period. The rate of elimination of hippuric acid after enteral ingestion of sodium benzoate was not markedly different from that obtained after intravenous injection. However, when glycocoll was administered with sodium benzoate *per os*, the rate of hippuric acid elimination was greatly increased. This increase in the rate of elimination might be considered to be due to (1) a stimulation of general metabolism as a result of the specific dynamic action of amino-acids, or (2) to an increase in the rate of absorption of sodium benzoate from the intestine under the influence of glycocoll, or (3) to an increased rate of synthesis of hippuric acid due to the presence of abnormally large quantities of glycocoll in the organism. Since alanine fed with the benzoate did not increase the rate of hippuric acid elimination, the effect is not considered to be due to a stimulation of general metabolism. Since the rate of elimination of hippuric acid was accelerated when sodium benzoate was injected *intravenously* and the glycocoll *fed*, the results can hardly be explained on the basis of an increased velocity of absorption of the benzoate from the intestine. It seems probable that an increase in the rate of synthesis has resulted from the presence in the system of extra glycocoll or its metabolites and that such glycocoll precursors are not furnished by alanine.

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[15] Kingsbury, F. B., and Swanson, W. W., *J. Biol. Chem.*, 1921, xlvi, p. iv; *Arch. Int. Med.*, 1921, xxviii, 220.

THE AMINO-ACID NITROGEN IN THE BLOOD AND ITS POSSIBLE RELATION TO THE ELEVATION OF THE METABOLISM IN MYELOGENOUS LEUCEMIA.

By K. SANDIFORD, W. M. BOOTHBY, and H. Z. GIFFIN.

(*From the Metabolism Laboratory, Mayo Clinic and Mayo Foundation, University of Minnesota, Rochester.*)

In a series of five cases of myelogenous leucemia total and basal metabolism experiments were carried out in conjunction with the study of the chemistry and morphology of the blood. The main point developed by this study was the frequent finding of an increase in the amino-acid partition of the non-protein nitrogen of the blood (Folin method), accompanied by an increase in the basal metabolism. The values ranged between 5 and 16 mg. and the average was 10 mg. (normal 5 to 7 mg.); the basal metabolic rate varied between +6 and +81 per cent. In all instances in which the amino-acids were increased there was some, but not necessarily a proportional, increase in the basal metabolism. On the other hand, as shown by four other cases in which only one determination was obtained the basal metabolism was distinctly elevated in two cases without an increase in the amino-acids. In three cases of lymphatic leucemia the amino-acids were 5, 6, and 9 mg. with a basal metabolic rate of +55, +3, and +25 per cent. In a case of polycythemia the amino-acids were 11 mg. and the basal metabolic rate was +21 per cent. Twenty-one patients with exophthalmic goiter and six with adenomatous goiter with hyperthyroidism showed no elevation of the amino-acids or other non-protein nitrogenous substances in the blood.

THE ANTIKETOGENIC ACTION OF GLUCOSE.

BY GEORGE ERIC SIMPSON.

(From the Biochemical Laboratory, Washington University School of Medicine, St. Louis.)

The urinary excretion of acetone bodies is determined for short intervals after the ingestion, by normal starving men, of (*a*) glucose alone, (*b*) hydroxybutyric acid alone, and (*c*) glucose and hydroxybutyric acid simultaneously. The antiketogenic effect of small quantities of glucose is at its height within a few hours after ingestion. The type of experiment exemplifies the procedure developed for a resurvey, now under way, of substances thought to possess antiketogenic action.

EXPERIMENTS ON THE UTILIZATION OF THE CALCIUM OF ALMONDS BY MAN.

BY MARY SWARTZ ROSE AND GRACE MACLEOD.

(From the Department of Nutrition, Teachers College, Columbia University, New York.)

Calcium balance experiments have been conducted on healthy young women taking for five consecutive 3 day periods a simple mixed diet in which blanched and finely ground almonds contributed from 73 to 86 per cent of the total calcium. The results, calculated for 1 kilo of body weight and averaged for the last 12 days in order to exclude fully the effect of previous diets, are shown in Table I.

With two exceptions the subjects were practically in calcium equilibrium. In these two the intake was probably too low to meet requirement. Three experiments were also conducted on a diet in which from 69 to 75 per cent of the calcium was derived from milk. The returns in terms of body weight were as given in Table II.

In Cases 1, 2, and 3 the calcium of almonds seems to have been utilized quite as well as the calcium of milk, five milk experiments (including the two previously reported) showing equilibrium on about 6 mg. per kilo. In the other almond experiments from 8 to 12 mg. appear to have been required to establish equilibrium. From these experiments the conclusion is reached that the calcium of almonds is not quite so effectively utilized as that of milk. Digestion experiments showed good utilization of the almond diet.

TABLE I.

Average Daily Intake and Output of Calcium on Almond Diet.

No.	Subject.	Body weight.	Ca from almonds.	Intake.	Output.	Balance.
		kg.	*per cent*	*mg. per kg.*	*mg. per kg.*	*mg. per kg.*
1	B. H.	45	74	4.1	5.0	−0.9
2	M. F.	60	73	4.1	6.0	−1.5
3	M. K.	60	73	4.1	4.9	−0.8
4	D. H.	75.8	73	8.8	7.7	+1.1
5	E. B.	55	85	9.4	9.4	
6	H. G.	55	85	9.3	11.6	−2.3
7	J. R.	58	85	9.3	11.0	−1.8
8	B. B.	52.7	86	5.5	10.0	−4.5
9	H. C.	50.5	86	5.7	7.1	−1.4

TABLE II.

Average Daily Intake and Output of Calcium on Milk Diet.

No.	Subject.	Body weight.	Ca from milk.	Intake.	Output.	Balance.
		kg.	*per cent*	*mg. per kg.*	*mg. per kg.*	*mg. per kg.*
10	B. B.	54	70	6.0	6.4	−0.4
11	G. C.	62.5	74	6.4	6.2	+0.2
12	C. M.	45	71	6.6	7.4	−0.8

THE UTILIZATION OF SUGAR BY RATS DEPRIVED OF VITAMINE B.

By H. A. MATTILL.

(From the Department of Vital Economics, University of Rochester New York.)

A single rat under a bell jar of 5 to 6 liters capacity produces during 10 to 15 minutes sufficient change (0.75 to 2 per cent) in O_2 and CO_2 content of the air to give satisfactory respiratory quotients by means of the Haldane apparatus. Air is withdrawn over mercury through a tube in the top of the bell jar. The stopper also carries a thermometer and a small mercury manometer, but the data on total heat production show wide variations due to the movements of the animal. The accuracy of the respiratory quotients, however, is satisfactory. Alcohol checks can be run in 1 to 2 minutes, depending on the size of the flame employed.

The respiratory quotients on a synthetic ration containing casein, starch, lard, salts, butter fat, and yeast varied from 0.80 to 0.90. Basal quotients usually showed less variation and averaged 0.75 to 0.76. On the synthetic ration without yeast, the quotients were usually lower than those of the controls, and approached the basal values; this was to be expected as a result of the decreased food consumption. Ingestion of cane-sugar or glucose was followed by a rise in the quotient to 0.90 and beyond in both sets of animals, thus indicating that deprivation of vitamine B does not interfere with the processes of glucose combustion.

STABILITY OF VITAMINE IN COD LIVER OIL.

BY H. STEENBOCK, J. H. JONES, AND E. B. HART.

(From the Department of Agricultural Chemistry, University of Wisconsin, Madison.)

Puppies at an age of 6 weeks on a ration of cooked white cornmeal and oat meal *ad libitum* and 5 gm. of casein, 2 gm. of sodium chloride, 5 gm. of calcium phosphate, and 200 cc. of skimmed milk per day were given in addition 5 cc. of cod liver oil or the unsaponifiable material obtained from 5 gm. of cod liver oil daily. Such dogs showed normal growth, normal Ca and P in the blood serum, normal ash content of the bones, and normal distribution of inorganic materials in bone as determined by x-ray examination.

Without the cod liver oil adjuvant, saponified or not, growth was inhibited, tetany resulted, Ca and P in serum were much reduced, and bones were poorly calcified as shown by ash content and x-ray examination.

THE NEPHROPATHIC ACTION OF MUCIC ACID.

By WILLIAM C. ROSE and PAULINE S. DIMMITT.

(From the Laboratories of Physiological Chemistry of the University of Texas, Galveston, and of the University of Illinois, Urbana.)

In continuation of studies reported a year ago[16] on the nephropathic action of dicarboxylic acids, recent experiments indicate that mucic acid, when administered subcutaneously as the sodium salt in doses of 0.5 gm. or more, produces in rabbits a severe nephritis. Marked retention of non-protein nitrogen, urea, and creatinine occurs, while the phenolsulfonephthalein elimination may fall almost to zero. Microscopic examination of the kidneys shows that the lesions involve the tubules primarily, with more or less secondary involvement of the glomeruli. In some cases hemorrhagic areas may be observed in the cortex.

Adipic acid, on the other hand, is only slightly nephropathic. These data, together with those formerly reported, seem to indicate that the introduction of hydroxyl groups in dicarboxylic acids greatly increases their renal toxicity. Both tartaric and mucic acids are much more nephropathic than are the corresponding succinic and adipic acids.

Preliminary experiments on the oral administration of mucic acid indicate that, as in the case of tartaric acid, very large doses are necessary to produce nephritis in rabbits. We are extending these studies to other acids.

THE ENZYMES OF THE RED BLOOD CORPUSCLES OF THE MAMMAL.

By WITHROW MORSE.

(From the Department of Physiological Chemistry, School of Medicine, West Virginia University, Morgantown.)

We have previously reported that erythrocytes of the mammal exhibited no autolysis even after long periods. We wish to extend these observations to include other enzymes. The blood of the dog in addition to the absence of proteoclastic enzyme capable of digesting the tissues of the corpuscles containing hemoglobin, shows absence of amylase, esterase, and invertase, but positive findings were obtained for lipase and catalase.

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[16] Rose, W. C., *J. Biol. Chem.*, 1922, l, p. xxiii.

With a view to determining the rôle which the presence of a nucleus may have in regard to the compliment of enzymes in erythrocytes, we studied the enzyme content of the blood of *Cryptobranchus alleghaniensis*, a salamander having nucleated corpuscles, but we were unable to detect any difference in suite of enzymes from that of the enucleated types of the mammal, catalase and lipase being found as in the blood of the dog.

STUDIES ON THE METABOLISM IN PREGNANCY.

I. CHANGES IN THE TENSION OF ALVEOLAR CARBON DIOXIDE.

By ALLAN WINTER ROWE.

(From the Department of Chemistry, Evans Memorial Hospital, Boston.)

A preliminary report of one phase of study of gaseous metabolism in pregnancy; two groups of cases studied, one from a prenatal out-patient service of a large metropolitan hospital, the other from a nursing home for unmarried mothers.

The Marriott and Fredericia methods were compared and the latter was selected as more convenient and less liable to error. Duplication of the respiratory phase was shown to be possible with the variation of ± 1.3 mm. Fredericia and Haldane were compared, using a Fredericia apparatus with a 3-way intake valve and capillary delivery as collection apparatus. Displacements were made over mercury. Deviations were less than ± 2 mm. in twenty-one series with a mean of — 0.4 mm. Haldane and Fredericia were compared in the same sample of air, collecting in the modified Plesch-Higgins collecting bag and passing air for Haldane through the modified Fredericia apparatus. Deviations in fifteen series were the same as in the preceding group with an average of ± 0.

In 56 cases of normal pregnancy carried to term with 310 observations, the average tension of alveolar carbon dioxide was 32.6 mm. In 13 cases with 45 observations of ketosis the average was 30.9 mm. In 14 cases with 61 observations of grave pathological states without ketosis the average was 32.8.

In 22 normal cases with 135 observations, the total acid, as estimated from the ammonia and titratable acid elimination, was 790 cc., an entirely normal figure. In 18 normal cases, the average ammonia output was 0.54 gm.; the phosphoric anhydride, 1.88; and the alveolar carbon dioxide, 29.9 mm. In another series of 10 cases the figures were 0.45, 1.75, and 33.7 mm.

The ammonia is normal, the phosphates show the possibility of slight phosphate retention, the alveolar carbon dioxide is definitely subnormal. There is no certain evidence of acid retention. Vital capacity measurements show no diminution, and in many cases an increase during pregnancy. Indirect methods had to be used due to the impossibility of obtaining blood samples.

To conclude, evidence of a marked lowering of the tension of alveolar carbon dioxide in normal pregnancy is presented comparable to that observed in well defined ketosis.

SOME CHEMICAL REACTIONS OF INSULIN.

By C. H. BEST and J. J. R. MACLEOD.

(From the Department of Physiology, University of Toronto, Toronto, Canada.)

Every sample of insulin prepared by the usual method from the pancreas of the ox or pig that has been examined by us, has given a decidedly purple color by the biuret test, an orange color by the xanthoproteic test, and a faint purple (masked by browning) by the Hopkins-Cole reaction. The intensity of the reactions has varied considerably with different preparations. On the other hand, insulin prepared from the pancreas of the skate by alcoholic extraction and heat gives none of the color reactions for protein. That the trace of protein in ox insulin preparations is an impurity is evidenced by the fact that after precipitation with phosphotungstic acid and removal of the latter by ether and barium, the preparation retains its powers to cause hypoglycemia.

Insulin gives none of the usual color reactions for epinephrine.

Moderate heat does not appreciably affect the strength of insulin, at least in faintly acid reaction (pH between 5 and 6). Thus, it may be heated on a boiling water bath for at least 10 minutes, and probably much longer, without perceptible alteration in strength, but it is destroyed by actual boiling for 3 minutes. Acidified hot water may therefore be used for the extraction of insulin from the pancreas.

Moderate degrees of alkalinity (to litmus) do not affect the strength of insulin during a period of 6 minutes at room temperature. We cannot as yet state the degree of alkalinity which insulin can withstand.

Insulin in acid reaction is adsorbed by kaolin, charcoal, etc. Thus, after shaking 4 cc. of insulin with kaolin for 10 minutes, 0.8 cc. lowered the blood sugar from 0.104 to 0.060 in 1 hour, without symptoms, whereas 0.8 cc. of the original insulin lowered it from 0.098 to 0.037 in 1 hour, and caused convulsions.

It has so far been impossible to demonstrate any dialysis of insulin through collodion sacs, and usually there is also a marked reduction in the strength of insulin within the dialyser. These experiments have been conducted either by frequently changing the water outside the dialyser or by using a small volume of unchanged water. Dialysis has been allowed to proceed for 3 hours.

On several occasions it has been found that the potency of the insulin preparations is greatly reduced by passing through a Berkefeld filter. Thus:

1 cc. of ox insulin before being passed through a Berkefeld filter lowered the blood sugar in 2 hours from 0.126 to 0.042, causing convulsions, and after it, from 0.130 to 0.072 with no symptoms.

3 cc. of skate insulin before being passed through a Berkefeld filter lowered the blood sugar in 2 hours from 0.120 to 0.054, causing convulsions, and after it from 0.122 to 0.090, with no symptoms.

By the method of formol titrations (Sörensen) it has been found that proteolytic enzymes are present in extracts of ox pancreas after the removal of the alcohol by the method described elsewhere, but none has been detected in the final preparations of insulin.

THE INFLUENCE OF PANCREATIC PERFUSATES ON THE BLOOD SUGAR, D:N RATIO, AND RESPIRATORY QUOTIENT OF DEPANCREATIZED ANIMALS.

By HARRY D. CLOUGH, ARTHUR M. STOKES, C. B. F. GIBBS, NEIL C. STONE, and JOHN R. MURLIN.

(From the Physiological Laboratory, University of Rochester, Rochester.)

Perfusion, by Clarke's method, of the pancreas of the dog, pig, and cow extracts the antidiabetic substance in sufficient concentration to affect favorably all the major symptoms of diabetes produced by depancreatization. In several instances the blood sugar was reduced to normal or lower by a single injection; the excretion of sugar is stopped by two or three injections; and the respiratory quotient may be raised at will by repeated injections. When the perfusion fluid is made slightly acid more potent extracts are obtained. The name *glucopyron* is suggested as the one most appropriate for the active antidiabetic substance however obtained from the pancreas. There is no evidence that it is a *chalone* in Schafer's sense: hence the name *insulin* suggested by him is inappropriate.

SOME PROPERTIES OF AN ACTIVE CONSTITUENT OF PANCREAS (INSULIN).

BY E. A. DOISY, MICHAEL SOMOGYI, AND P. A. SHAFFER.

(From the Laboratory of Biological Chemistry, Washington University School of Medicine, St. Louis.)

Insulin has been prepared from beef pancreas by modifications of methods outlined by Banting and Best and by Collip. More than fifty different preparations have been made and used in work with dogs and rabbits, in *in vitro* experiments, and with human diabetics, with marked success.

The active material has been purified and concentrated to the point that about 0.25 mg. of substance produces marked hypoglycemia and characteristic convulsions in rabbits weighing about 1 kilo. This material is a white powder having the following properties. It contains about 14 per cent nitrogen, and is free from phosphorus; it gives distinct biuret reaction, faint reaction with glyoxylic acid, and doubtful Millon's reaction. Its solutions are levo-rotary. Its activity in large part withstands boiling in 0.1 N HCl for 5 minutes. It is freely soluble in water except at C_H about 5 to 6, at which reaction it is precipitated (incompletely) from its solutions. It is soluble also in alcohol except at its isoelectric point. It is fairly completely precipitated from water solutions by one-half saturation ammonium sulfate. The substance appears to be an albumose or globulin. Although there appears to be considerable uniformity in the relation between activity and weight of substance, in various purified preparations made in different ways, the identity of the active substance cannot be regarded as established. The possibility remains that the protein described contains the active material as admixture.

The method of preparation of solutions suitable for experimental work which we have found the most satisfactory is briefly as follows:

For each kilo of finely ground fresh pancreas hash add about 40 cc. of 10 N H_2SO_4 (or 40 cc. concentrated HCl), 1,200 cc. of 95 per cent alcohol, and 300 cc. of water. Stir well at intervals during 4 to 12 hours, at room temperature. Strain through cloth and press out liquid as completely as possible. Reextract hash with a liter or more of 60 per cent alcohol, and again strain and press out liquid. To the combined very turbid liquid add NaOH to faint acid reaction to litmus. Filter through paper, and evaporate filtrate placed in shallow trays in a warm air current (temperature of solution 20 to 30°C.) until all odor of alcohol is gone, or until the volume is reduced to one-quarter to one-tenth the original volume. Transfer the liquid without filtering to a separatory funnel, rinsing the dishes with small amounts of water. Acidify slightly with HCl or H_2SO_4, add 40 gm. of solid $(NH_4)_2SO_4$ for each 100 cc. of solution and shake until dissolved. On standing a few hours the flocculent precipitate first formed rises to the surface and coagulates to a compact layer. Separate the lower liquid as completely as possible, leaving the precipitate in the funnel to drain. When all possible liquid has drained off, add to funnel 75 per cent alcohol (about 50 cc. for each kilo of pancreas hash used), which dissolves the insulin. Rinse the solution and insoluble residue into a centrifuge tube with small amounts of 60 per cent alcohol, and centrifugate. Pipette off the clear alcohol solution and add 8 to 10 volumes of 95 per cent or absolute alcohol. Adjust the reaction of the mixture to C_H 5 to 6 and filter after standing some hours. The precipitate is dissolved in water with the addition of small amounts of either acid or alkali. We ordinarily obtain one "1 kg. rabbit unit" from each 2 gm. of pancreas hash used. Occasionally a better yield has been obtained. For use in human diabetes the material should be purified by means of repeated precipitation at the isoelectric point, and by precipitation by ammonium sulfate, and by alcohol.

NITROGEN DISTRIBUTION IN THE BLOOD AND URINE OF THE ALLIGATOR.

By ALEITA HOPPING and ERNEST L. SCOTT.

(From the Department of Physiology, Columbia University, New York.)

Unlike birds and some reptiles, alligators excrete very little uric acid. Blood of the alligator contains from 1.8 to 2.6 mg. of uric acid per 100 cc. of blood, about the same value for human blood. In the urine, uric acid represents from 10 to 20 per cent of the total nitrogen. Uric acid is evidently not the main end-product of nitrogen metabolism in these reptiles. In the urine, ammonia varies from 53 to 80 per cent of the total nitrogen. In one alligator the total nitrogen was 215 mg., the ammonia nitrogen, 171 mg. (79 per cent), urea nitrogen, 7 mg. (3 per cent), and uric acid nitrogen, 40 mg. (18 per cent), per 100 cc. After sterile catheterization, the amount of urea nitrogen gradually increases. In the alligator the cloacal chamber is separated by a sphincter from the intestine and fulfills the function of a bladder. It is possible that decomposition of nitrogenous waste products may occur here. The nitrogen distribution in the blood is being studied and may throw light on this question.

DYE-PROTEIN AGGREGATES.

I. CONGO FIBRIN.

By L. F. SHACKELL.

(From the Department of Pharmacology and Therapeutics, College of Medicine, University of Illinois, Chicago.)

A special preparation of Congo fibrin was made by adding an excess of Congo red to a clear hot solution of washed fibrin in 0.1 N NaOH. Neutralization produced a precipitate of dyed *m*-protein. This was coagulated by boiling while in suspension in a saturated solution of NaCl. The excess of dye was removed by repeated washing with boiling distilled water. The Congo fibrin was dried *in vacuo* over sulfuric acid, pulverized, and passed through a 100 mesh sieve.

A suspension of this Congo fibrin in glycerol has yielded no free Congo red to the glycerol in 2 years. A suspension in distilled water yields up no color in a month when kept at 10°C. The material is rapidly and completely digestible by pepsin in acid solution. The individual particles range in size from 0.01 to 0.25 mm.

Portions of the glycerol suspension have been fractionated by centrifugation so that the range of size of particles in each fraction was fairly small. The velocity of peptic digestion of the largest particles as compared with that of the smallest was much faster than would be expected if digestion were to take place only at the surfaces of the particles. The velocity of digestion of the original suspension was strikingly similar—except in its initial stage—to that of a monomolecular reaction. The graph of the process is, however, simply a skew variation curve. The view is advanced that the monomolecular formula can be applied to protein hydrolyses only by neglecting the details of the earlier stages. A skew variation curve, on the other hand, may mean simply the rapid digestion of numbers of small colloidal particles accompanied by a more leisurely degradation of the larger ones.

THE EFFECT OF INJECTIONS OF VARIOUS SUBSTANCES UPON THE BLOOD COMPOSITION OF THE TORTUGAS CRAWFISH PANULIRUS ARGUS.

By SERGIUS MORGULIS.

(*From the Department of Biochemistry, College of Medicine, University of Nebraska, Omaha.*)

The study of the non-protein components of arthropod blood has led to the conclusion that the composition varies rapidly with the changes in the nutritive condition of the animal. A series of experiments was performed with the large crawfish, *Panulirus*, from which blood can be drawn several times without causing the animal any apparent injury. Four substances were studied: glucose, sucrose, urea, and ammonium sulfate. These were dissolved in sea water and injected deep into the thoracic muscles. Blood samples were obtained from a slit in a caudal fin, and were taken prior to the injection and at various times afterwards. Ammonium sulfate was found rather toxic (effect of acidity?) but 0.5 gm. could be tolerated by the animal. This substance gets into the blood almost at once; it has been recovered about ½ hour after the injection. It is, however, very rapidly excreted and 2 hours later it is completely eliminated and the total non-protein N of the blood is usually diminished as compared with the original content. The behavior towards urea injections is quite different. The largest dose given at one time was 1 gm., but there is good reason to believe that much greater quantities might be given without causing any toxic effects. Striking changes in the blood follow such an injection. A great increase in the non-protein N and in the urea N of the blood occurs, reaching a maximum in about 2 hours. At that time an increase by as much as 70 mg. was sometimes found. The N content of the blood filtrates then commenced to diminish and the original non-protein N level was reached in 12 to 14 hours after the injection. The injection of the urea was always associated with an increase in the uric acid content of the blood. The changes in the uric acid content ran more or less parallel to the changes in the N. Working with crawfish which have been already 1 or 2 days in captivity the change in the uric acid content was particularly striking as the control sample of blood was usually free, or practically so, from uric acid and there was no color, or only the faintest tinge of blue, produced, but the samples obtained 2 to 3 hours after the injection gave intense color reactions. Glucose was injected in quantities ranging from 0.4 to 0.8 gm. The quantity was limited entirely by considerations of convenience of analysis and not by the tolerance which is undoubtedly much greater than 0.8 gm. Temporary hyperglycemia is produced by these injections, and 2 hours after an injection increases in the blood sugar of 155 to 175 mg. were found. The blood sugar curve after reaching a maximum, begins to slope off again and the original sugar level is usually reestablished in 8 to 12 hours. Very interesting changes follow an injection of sucrose. This was injected in doses of 0.4 to 0.5 gm. Within less than 1 hour after the injection the reducing sugar in the blood increases in amount. The blood is also loaded with sucrose as can be shown by the very great increase in hydrolyzable sugar in the blood filtrates. It seems from the experimental data that after the 1st hour the levels of reducing sugar and of hydrolyzable sugar in the blood behave reciprocally. In one crawfish this was followed for 93 hours after the injection. The animal remained hyperglycemic until almost the very end of this experiment. After 93 hours the original sugar level had been reestablished though there was still 8 mg. of hydrolyzable sugar per 100 cc. of blood.

A STUDY OF THE BLOOD OF THE CRAWFISH PANULIRUS ARGUS WITH SPECIAL REFERENCE TO THE ABSENCE OF CREATININE IN ARTHROPOD BLOOD.

By SERGIUS MORGULIS.

(*From the Department of Biochemistry, College of Medicine, University of Nebraska, Omaha.*)

The non-protein constituents of the crawfish blood were studied by the Folin-Wu blood analysis procedure, except for the uric acid determinations which were made according to Benedict's new method. The findings on this arthropod from Tortugas agree well with the results already reported here previously[17] on the blood of several arthropods from Woods Hole. In a large number of animals examined immediately after capture the following variations were found:

	Per 100 cc. of blood.			
	Sugar.	Non-protein N.	Urea N.	Uric acid.
	mg.	*mg.*	*mg.*	*mg.*
Maximum........................	71	29	11	2.0
Minimum........................	19	15	6	0.3

Owing to the fact that these crawfish could be bled repeatedly, the question of the influence of the nutritive condition of the animal upon the non-protein composition of the blood could be studied on the same animal. In one instance the changes were observed for 44 hours after the animal had been brought to the laboratory and the results were as follows: the sugar content at the end of 24 and 44 hours changed from the original level of 71 to 18 and 13 mg., respectively; the non-protein N changed from 16 to 14, then to 10 mg.; the uric acid content from 0.7 mg. diminished to 0.3 mg. at the end of 24 hours, and was entirely absent after 44 hours. The complete disappearance of the uric acid from the blood has often been noted to occur in a much shorter time.

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[17] Morgulis, S., *J. Biol. Chem.*, 1922, l, p. lii.

The blood filtrates were studied especially with the view of determining if they contain any creatinine. The blood of *Panulirus* at no time showed any trace of this substance and the color developed by the blood filtrates could be read against a blank containing water provided the colors were matched within 15 minutes. The same was also observed in the case of several other arthropods. References in the literature to creatinine in arthropod blood notwithstanding, it can be stated definitely and with the support of an extensive number of analyses that creatinine is never present in the blood of arthropods.

THE ISOLATION OF BILIVERDIN FROM BILE.

By WILLIAM M. BARRY and VICTOR E. LEVINE.

(*From the Department of Biological Chemistry, School of Medicine, Creighton University, Omaha.*)

To each liter of ox bile are added 50 cc. of 10 per cent HCl. The bile is then placed in a large beaker, left uncovered, and exposed to sunlight until dark green. The acid precipitates the mucin-like substances and nucleo-albumins. The sunlight catalyzes the oxidation of bilirubin to biliverdin. The bile is then carefully decanted, warmed, and extracted with chloroform to remove the cholesterol. It is then extracted with ether until free from ether- and chloroform-soluble substances. To the liquid remaining after this treatment are added 50 cc. of 10 per cent HCl, which precipitates the pigment and the bile acids. The supernatant liquid is drawn off and rejected.

The viscous portion containing the pigment is warmed on a water bath until all the odor of chloroform has disappeared and is then made up to five times its volume with 95 per cent ethyl alcohol. This solution is then diluted with one and a half times its volume of distilled water. The pigment which settles out is washed repeatedly with distilled water, dried, and then washed with ether and again dried.

The pigment prepared thus is an amorphous green solid, insoluble in water, ether, and chloroform, but soluble in glacial acetic acid and in 95 per cent alcohol. It is found to be free from iron, sulfur (absence of the green pigment, choleprasin), fat, cholesterol, lecithin, and bile salts. The yield is about 2.5 gm. per liter of bile.

THE OCCURRENCE OF KETONE BODIES IN THE URINE OF NORMAL RABBITS IN A CONDITION OF HYPOGLYCEMIA FOLLOWING THE ADMINISTRATION OF INSULIN—A CONDITION OF ACUTE ACIDOSIS EXPERIMENTALLY PRODUCED.

By J. B. COLLIP.

(*From the Department of Biochemistry, University of Alberta, Edmonton, Canada.*)

The causation of the convulsive state in normal rabbits rendered hypoglycemic by insulin administration—a condition first noted by Collip and Macleod while collaborating with Banting and Best—has been the subject of much study. It occurred to the writer that the glycogen-forming activity of the injected insulin might by lowering the sugar tension on the blood actually jeopardize the oxidation of glucose in the tissue even though a certain amount of sugar did still exist in the blood stream. If such were the case ketone bodies would undoubtedly be formed and the animal would manifest other signs of an acute acidosis.

The urine was therefore obtained from rabbits immediately following the convulsive state induced by insulin, and in every instance a decided positive Rothera test was obtained. The ferric chloride test was not entirely satisfactory as a green precipitate was as a rule formed at first. On filtering, however, a port wine color was usually manifested in the filtrate. Later the urine was expressed from the bladder of rabbits after they had been injected with insulin, but before the blood sugar level had been reduced to the critical level of 0.045 per cent. A positive Rothera test was obtained in animals with blood sugar levels ranging from 0.065 to 0.045 per cent. Quantitative estimation of ketone bodies by the Van Slyke method indicated from 50 to 100 mg. per 100 cc. in samples obtained after the convulsive state had become manifested.

It has also been noted in some cases that as the level of blood sugar falls the CO_2-combining power of the blood falls progressively. In the convulsive state this factor may be as low as 28. If sugar be administered and the animal allowed to recover, the CO_2-combining power of the blood returns to approximately the original level within a day or two, and the urine also becomes free from ketone bodies. In other instances, however, little or no reduction in the CO_2-combining power of the blood was observed to occur.

These findings are of the greatest interest in that it would appear that while we have in insulin a substance which will correct all the acidotic signs both in experimental animals rendered diabetic, and in patients suffering from diabetes (if the dosage is adequate), it will, nevertheless, produce many of the cardinal symptoms of acidosis when administered to the normal animal in amount sufficient to produce a hypoglycemia of a marked degree.

THE DEMONSTRATION OF AN INSULIN-LIKE SUBSTANCE IN THE TISSUES OF THE CLAM (MYA ARENARIA).

By J. B. COLLIP.

(From the Department of Biochemistry, University of Alberta, Edmonton, Canada.)

When it was shown by the writer in collaboration with Banting, Best, and Macleod that the administration of insulin to a diabetic dog enabled the liver to store glycogen in large amount (25.6 per cent was observed in one instance), it was predicted that wherever glycogen occurs there would be insulin not far distant. The clam was chosen for investigation on account of its high glycogen content, over 11 per cent of glycogen having been recently found in the wet tissue of *Mya arenaria* by Collip.[18]

The method developed by Collip for the isolation of insulin in semipure form from the pancreas of the ox was applied with success to clam tissue. In one experiment an extract so prepared produced typical convulsions in a normal rabbit in 6 hours. The blood sugar at this time was 0.045 per cent and the convulsions were relieved by the subcutaneous injection of dextrose.

Latterly, it has been found that extracts made from clam tissue by a modified process manifest delayed action when administered to normal rabbits. Hypoglycemia of a marked degree is not manifested until the 2nd or 3rd day following the administration of the extract, whereas in certain instances an actual hyperglycemia may be noted for a few hours following the injection.

[18] Collip, J. B., *J. Biol. Chem.*, 1921, xlix, 297.

THE ORIGINAL METHOD AS USED FOR THE ISOLATION OF INSULIN IN SEMIPURE FORM FOR THE TREATMENT OF THE FIRST CLINICAL CASES.

By J. B. COLLIP.

(From the Department of Biochemistry, University of Alberta, Edmonton, Canada.)

The method applied in the preparation of the first insulin used in the treatment of clinical cases was developed by the writer during December and January last. In the critical first few weeks of clinical trial of insulin the preparation of the extract was carried out exclusively by the writer.

This method which is outlined below yielded excellent results at first when small quantities of material were being used; but as the "plant" was gradually enlarged the yield of potent material became less and less. Various modifications of the original method have since been made but it is of interest to note that the general principles developed in the first instance still apply in any modified process. It is now possible by a variety of ways to produce extracts containing the potent principle which are practically protein-, lipoid-, and salt-free. Some of these methods will be described in a subsequent communication. The original method was as follows:

To a small volume of 95 per cent ethyl alcohol, freshly minced pancreas was added in equal amount. The mixture was allowed to stand for a few hours with occasional shaking. It was then strained through cheese-cloth and the liquid portion at once filtered. The filtrate was treated with 2 volumes of 95 per cent ethyl alcohol. It was found by this treatment that the major part of the protein was removed while the active principle remained in alcoholic solution.

After allowing some hours for the protein precipitation to be effected the mixture was filtered and the filtrate concentrated to small bulk by distillation *in vacuo* at a low temperature (18 to 30°C.). The lipoid substances were then removed by twice extracting with sulfuric ether in a separating funnel and the watery solution was returned to the vacuum still where it was further concentrated till it was of a pasty consistency. 80 per cent ethyl alcohol was then added and the mixture centrifuged.

After centrifuging, four distinct layers were manifested in the tube. The uppermost was perfectly clear and consisted of alcohol holding all the active principle in solution. Below this, in order, were a flocculent layer of protein, a second clear watery layer saturated with salt, and a lowermost layer consisting of crystals of salt. The alcohol layer was removed by means of a pipette and was at once delivered into several volumes of 95 per cent alcohol, or better, of absolute alcohol. It was found that this final treatment with alcohol of high grade caused the precipitation of the active principle along with adherent substances. Some hours after this final precipitation the precipitate was caught on a Buchner funnel, dissolved in distilled water, and then concentrated to the desired degree by use of the vacuum still. It was then passed through a Berkefeld filter, sterility tests were made, and the final product was delivered to the clinic.

The essential points relating to the extract prepared as outlined above are: (1) It contains only a minimum of protein. (2) It is practically salt-free and can readily be made isotonic. (3) It is lipoid-free. (4) It is almost free from alcohol-soluble constituents. (5) It can be administered subcutaneously without fear of any local reaction.

Note.—This method was developed by the writer while he was attached to the Department of Pathological Chemistry, University of Toronto.

STUDIES ON BY-PRODUCT YEAST.

By JOSEPH S. HEPBURN.

(*From the Constantine Hering Laboratory, Hahnemann Medical College, Philadelphia.*)

Thoroughbred albino rats have been fed a diet consisting of purified foods to which dried yeast has been added as the sole source of the water-soluble B vitamine. Three grades of by-product yeast have been used: (1) bottom yeast, derived from manufacture of industrial alcohol from waste molasses; (2) brewery yeast; and (3) ale yeast.

All three grades were dried by the Wittemann process. The rats have grown and reproduced, and the offspring have likewise gained in weight. When the dried yeast from the industrial alcohol plant was included in the ration fed to immature chickens, they showed excellent gains in weight.

THE TITER VALUE OF CHICKEN FAT.

By JOSEPH S. HEPBURN.

(From the Constantine Hering Laboratory, Hahnemann Medical College, Philadelphia.)

The titer test was applied to twenty composite samples of abdominal fat from the common fowl, *Gallus domesticus*. The titer of the isolated insoluble fatty acids ranged between 32.50 and 37.60°C., the average for the twenty samples being 34.94°C.

A NOTE ON THE INITIAL ACIDOSIS OCCURRING WITH ANESTHESIA.

By GLENN E. CULLEN and J. HAROLD AUSTIN.

(From the John Herr Musser Department of Research Medicine, University of Pennsylvania, Philadelphia.)

In attempting to determine the mechanism of the marked acidosis which occurs with ether anesthesia and which is indicated by increase in the hydrion concentration, and decrease in the alkaline reserve of the blood, studies have been carried out with inhalation of other anesthetics as well as with other gases.

In previous studies we have found the acidosis existing ½ hour or more after commencement of the anesthesia.

In our present investigation we have made repeated observations during the first few minutes after commencing administration of the anesthetic and have found, within 5 minutes, an increase in the hydrion concentration and a decrease in alkaline reserve comparable with that present at the end of an hour.

Moreover, we have observed this initial acidosis after inhalation not only of the anesthetics, ether, chloroform, and nitrous oxide, but also of formaldehyde, pure nitrogen, and pure oxygen.

THE INFLUENCE OF ALKALI THERAPY ON KETOSIS IN DIABETES MELLITUS.

By HERMAN O. MOSENTHAL and JOHN A. KILLIAN.

(From the Departments of Medicine and of Biochemistry, New York Post-Graduate Medical School and Hospital, New York.)

Six patients confined in the Metabolic Ward were placed upon a standard diet comprising about 30 gm. of carbohydrate, 130 gm. of fat, and 40 gm. of protein, giving 1,400 to 1,500 calories. A preliminary period of 7 to 10 days permitted an adjustment of the patient's metabolism to this diet. During the experimental period, sodium bicarbonate was given by mouth in doses of 30 to 45 gm. per day until it was felt that the maximum effect of the alkali had been obtained. The after period was extended until a full recovery from the influence of the alkali was noted. In some instances two experimental periods were employed. Observations were made upon the sugar, acetone bodies, and carbon dioxide-combining power of the blood; and the total nitrogen, sugar acetone bodies, and reaction of the urine. Van Slyke's method was used for the determination of the acetone bodies. It was noted that the administration of the alkali produced a decrease in the acetone bodies of the blood associated with a rise in the carbon dioxide-combining power, and a drop in the concentration of sugar. At the beginning of the period of alkali therapy there was an initial rise in the acetone bodies of the urine but subsequently they fell, to within normal limits in some cases. The sugar excretion in the urine was also markedly decreased. In some instances the effect of the alkali therapy persisted for about 10 days after the sodium bicarbonate had been discontinued.

A SIMPLE METHOD OF ESTIMATING THE SALIVARY UREA.

By HERBERT W. SCHMITZ.*

(From the Department of Biochemistry, New York Post-Graduate Medical School and Hospital, New York.)

Since the concentration of the urea in the saliva is practically equal to that in the blood, the utilization of salivary urea estimations in determining the degree of urea retention has been recommended in a previous paper.[19] A simple method for estimating the salivary urea is described.

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* Medical Fellow of the National Research Council.

Technique: Into a large test-tube (graduated at 15 and 20 cc.) are introduced 2 cc. of filtered saliva, 2 drops of molecular phosphate, and 1 cc. of a 5 per cent urease solution. The tube is incubated in a beaker of water at 50°C. for 15 minutes. At the end of this time distilled water is added to the 15 cc. mark, 2 cc. of dialyzed iron (Merck's, 5 per cent Fe_2O_3), 1 cc. of a 20 per cent sodium sulfate solution, and distilled water to the 20 cc. mark. After the mixture has been shaken vigorously, it is poured upon a folded filter. 10 cc. of the filtrate (equal to 1 cc. of saliva) are now pipetted into a graduated cylinder and 5 to 10 cc. of dilute Nessler's solution, dependent on the content of nitrogen, are dumped in at once while the solution in the cylinder is rotated until the maximum color development is obtained. If a large amount of urea is present, sufficient distilled water is added until the color of the unknown approximates the color intensity of the standard. The colors are matched immediately in the colorimeter.

The results obtained with this method agree very favorably with those obtained with the aeration procedure.

A PHYSICOCHEMICAL METHOD OF CHARACTERIZING PROTEINS.

IV.

By EDWIN JOSEPH COHN.

(From the Laboratory of Physical Chemistry, Harvard Medical School, Boston.)

In previous reports to this society we have pointed out: (1) that the differences in the solubility of proteins that have led to their classification depend in large part upon their dissociation at their isoelectric points; (2) that the solubility of each protein that we have purified of its soluble compounds was constant at its isoelectric point; and (3) that this solubility represented the sum of the concentrations of the undissociated protein molecule and of the ions into which it dissociates.

By studying solubility in systems containing a protein and small amounts of its soluble compounds, we have succeeded in estimating the degree of dissociation of certain proteins at their isoelectric points. The methods that we have employed have been developed from the fundamental equations for the dissociation of amphoteric electrolytes. Their consideration has also yielded a graphical method of analyzing the solubilities of proteins over a small range—where they may be considered as dissociating as monovalent acids and bases—in the neighborhood of their isoelectric points.

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[19] Schmitz, H. W., *J. Lab. and Clin. Med.*, 1922, viii, 78.

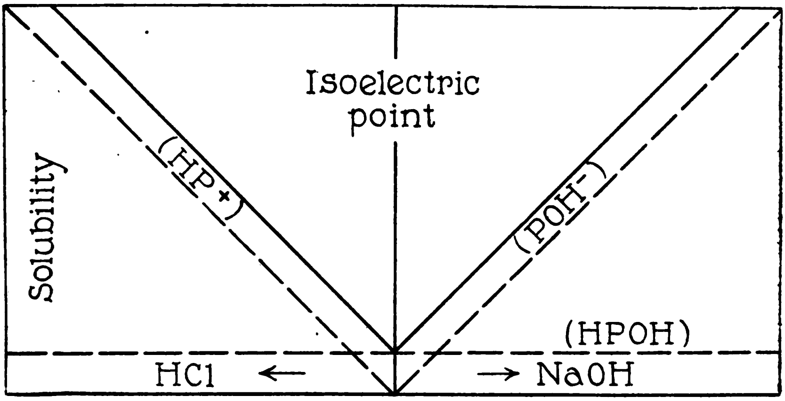

Fig. 1.

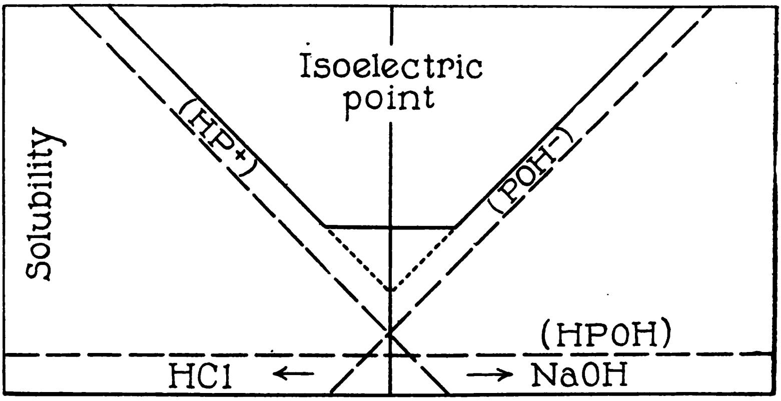

Fig. 2.

The solubility relations of two hypothetical proteins are represented in Figs. 1 and 2. The ordinates designate concentrations of the undissociated molecule (HPOH) and of the basic (HP^+) and the acidic (POH^-) ions; the abscissæ the excess of acid, or of base. The protein in Fig. 1 is not appreciably dissociated at its isoelectric point. The concentration of ions formed by its dissociation is, therefore, proportional to the acid or base with which it combines. A straight line represents the constant concentration of the undissociated molecule. Irrespective of the value ascribed to the latter, the "curve" describing the solubility of such a protein, therefore, consists of two straight lines, diverging from the isoelectric point.

Curves describing the solubility of proteins have, however, generally passed through rounded minima in the neighborhood of the isoelectric points. This suggests that the proteins were in part dissociated. In Fig. 2 the hypothetical protein combines with acids and bases in the same ratio as the protein in Fig. 1. The protein is, however, a stronger acid and a stronger base, and is, therefore, partly dissociated at its isoelectric point. The value of the ordinate at the point of intersection of the (HP^+) and the (POH^-) lines is a measure of the concentration of these ions at the isoelectric point. If the concentration of the undissociated molecule is added to the concentration of these ions, the solubility of the protein in the system is obtained. This solubility passes through a wide minimum at the isoelectric point. The "solubility curve" becomes a straight line, however, at such a distance from the point that the concentration of one of the ions becomes negligible in comparison with the other.

Our experimental determinations of the solubility of casein in systems containing small amounts of sodium hydroxide fall on such a straight line. Extension of this straight line to its point of intersection with the isoelectric line yields the concentration of the undissociated molecule and the casein anion.

$$(HPOH) + (POH^-) = 0.085 \pm 0.005 \text{ gm. per liter at } 25°C.$$

But we have previously reported the solubility of isoelectric casein:

$$(HPOH) + (POH^-) + (HP^+) = 0.11 \pm 0.005 \text{ gm. per liter at } 25°C.$$

By difference (HP^+) equals 0.025±0.01, and since at the isoelectric point (HP^+) = (POH^-), the latter also equals 0.025; (HPOH) equals 0.06; and the degree of dissociation:

$$(HP^+)\ (POH^-):(HPOH)^2 = Ka.Kb:Kw$$

must lie between 0.1 and 0.4.

CONCERNING THE NATURE OF COHESION.

By A. P. MATHEWS.

THE METABOLISM OF CREATINE.

By S. R. BENEDICT and EMIL OSTERBERG.

THE RELATIONSHIP BETWEEN STRUCTURE AND CHEMICAL PROPERTIES OF SOME SUBSTANCES RELATED TO THYROXIN.

By E. C. KENDALL.

RATE OF AMMONIA LIBERATION IN TRYPTIC DIGESTION OF PROTEIN.

By ANDREW HUNTER and R. G. SMITH.

ON NORMAL BLOOD SUGAR VALUES IN MAN AND ANIMALS.

By W. S. McELLROY and H. O. POLLOCK.

THE PROTEIN REQUIREMENTS IN DIABETES.

By A. I. RINGER, S. BLOOM, and S. JACOBSON.

MINERAL DEFICIENCIES OF MILK AS SHOWN BY GROWTH AND FERTILITY OF WHITE RATS.

By AMY L. DANIELS and MARY K. HUTTON.

THE EFFECT OF HEAT TREATMENT OF MILK FEEDINGS ON THE MINERAL METABOLISM OF INFANTS.

By AMY L. DANIELS and GENEVIEVE STEARNS.

ABSTRACT OF THE EXECUTIVE PROCEEDINGS OF THE SEVENTEENTH ANNUAL MEETING.

New Members: E. J. Baumann, F. A. Cajori, G. W. Clark, G. R. Cowgill, J. M. Looney, J. H. Mueller, C. N. Myers, B. S. Neuhausen, Ruth E. Okey, H. B. Speakman, R. W. Thatcher, Chi Che Wang.

Clinical Membership: Amendments to the By-Laws providing for the election of Clinical Members were passed by the Society.

Clinical Members: J. H. Austin, W. R. Campbell, S. W. Clausen, J. L. Gamble, C. H. Greene, G. A. Harrop, Jr., Christen Lundsgaard, J. P. Peters, Jr., E. D. Plass, A. T. Shohl, W. C. Stadie, F. F. Tisdall.

Deceased Members:

Henry Prentiss Armsby.

Henry Prentiss Armsby, the foremost leader in the nutrition of animals in America, was born in Northbridge, Mass., September 21, 1853. After a training in analytical chemistry at Worcester Polytechnic Institute and graduate study at the Sheffield Scientific School, from which he received his doctorate in 1879, he began active work in the agricultural experiment stations of America and was one of that group of men who carried this whole general project through to its splendid success. Primarily a chemist, interested in agricultural and nutrition problems, it was in the fields of biophysics and physiology that he made perhaps his greatest contributions, for he established at State College, Pa., the large respiration calorimeter for animals, with which he began collecting a set of fundamental data which has made possible not only much further animal nutrition work but which particularly made his services of supreme importance on the Interallied Scientific Food Commission, on the National Research Council, and numerous other organizations. He was a master in the handling of the knowledge of transformations of energy. His catholicity of views may perhaps best be illustrated by the fact that, although by training a chemist, he was elected to the National Academy of Sciences in the division of physiology and pathology. But only those who had the privilege of knowing Dr. Armsby personally can begin to realize the wonderful charm, sweetness of disposition, modesty, insight, and keenness of perception of large problems. His presence and his wise counsels will never be forgotten. The overworked, frail constitution succumbed on October 19, 1921, and it is only with great difficulty that the scientific world can meet this loss.

FRANCIS G. BENEDICT
THORNE M. CARPENTER
Committee.

Edward Kellogg Dunham.

By the sudden death of Edward Kellogg Dunham, on April 15, 1922, in his sixty-third year, the American Society of Biological Chemists has lost one of its valued members. He was an original stockholder in the Journal of Biological Chemistry, treasurer, trustee for the Herter Memorial Endowment Fund, as well as editor for many years, and he gave freely of his time and energy in furthering the best interests of the Journal. His sage counsel and mature judgment in addition to more material and scientific contributions were always enlisted in our interest and no decision of any moment concerning the Journal's welfare was ever taken without his helpful cooperation. Those of us who feel pride in the present status of the Journal of Biological Chemistry will not readily forget how large a share of its successful development was due to Dunham's foresight and guidance, especially in its earlier and more difficult days.

While Dunham's main contributions to science were in bacteriology and pathology, his interest in chemical problems was early aroused and maintained throughout forty years of active professional life. As a student in the School of Mines of Columbia University he acquired a knowledge of chemical technique that later was constantly turned to good account. On the completion of his medical studies at Harvard he became one of the small band of pioneer workers who appreciated the significance of the developing science of bacteriology and studied for a considerable period in Koch's laboratory in Berlin. Among other important work of this period was his independent discovery of the familiar "cholera red" reaction. On his return to America he at first engaged in public health work in Massachusetts and later became professor of pathology in the Bellevue Medical College of New York University. This association was maintained until a few years ago when he became Emeritus Professor, although he continued active in promoting the work of the college and gave occasional lectures. Perhaps his most important work of this period was concerned with the meningococcus and the gas bacillus of Welch, and with the histology of tumors. Later he became interested in the chemistry of the lipoids found in glandular organs. A skillful and resourceful experimenter, many devices and laboratory aids now in every day use are due to his ingenuity.

At the entrance of the United States into the Great War, Dunham at once took up his duties in the Army Medical Reserve Corps and soon became especially interested in the study of empyema. He was appointed chief of an "Empyema Commission" by the Surgeon General and did most valuable work in improving the treatment of these cases. At the close of the war, having attained the rank of Lieutenant-Colonel, he continued these studies with unabated enthusiasm and indeed was busy on his empyema report for the "Medical History of the War" up to within a few minutes of his death. It is a satisfaction to know that this work, which is regarded by competent critics as of the first importance, was so far advanced that his devoted colleagues will be able to complete it, practically as the author would have wished.

But while Dunham's scientific attainments and breadth of knowledge were of an exceptionally high order, those who knew him quickly realized that he possessed other and rarer qualities. Seldom is it given to anyone to possess so great a faculty for inspiring affection and esteem in all around him. A spirit of sweet reasonableness pervaded his life, and strife was unknown to him. A wise friend and comforter to those in difficulty, his loss is a tragedy in the lives of many.

"While men strive and fail, and faint by the way, the work they have tried to do does not fail, but is eternally carried on in ways unknown to them."

H. D. DAKIN
GRAHAM LUSK
Committee.

OFFICERS FOR THE YEAR 1923: *President*, Philip A. Shaffer; *Vice-President*, Henry C. Sherman; *Secretary*, Victor C. Myers; *Treasurer*, Walter R. Bloor; *Additional Members of the Council*, Harold C. Bradley, Andrew Hunter, Albert P. Mathews., *Nominating Committee*, C. L. Alsberg, S. R. Benedict, Otto Folin, Walter Jones, E. C. Kendall, P. A. Levene, J. R. Murlin, F. P. Underhill, D. W. Wilson.

DUES: The dues for the year 1923 were fixed at $2.00.

ATTENDANCE: Sixty-nine members of the Society attended the meeting.

VICTOR C. MYERS,
Secretary.

PROCEEDINGS

OF THE

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

EIGHTEENTH ANNUAL MEETING

St. Louis, Missouri

December 27–29, 1923

ABSTRACTS OF PAPERS
ABSTRACT OF EXECUTIVE PROCEEDINGS

Volume VI
No. 2

PROCEEDINGS OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS.

EIGHTEENTH ANNUAL MEETING

St. Louis, Mo., December 27–29, 1923.

Abstracts of Scientific Proceedings........................ 3
Abstract of Executive Proceedings........................ 58

For the List of Members and the Constitution and the By-Laws of the Society see the 1924 Register of the Federation of American Societies for Experimental Biology, comprising the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, and the American Society for Experimental Pathology.

Reprinted from The Journal of Biological Chemistry
Vol. LIX, pp. iii-lvii, 1924 (February).

THE PRODUCTION AND PRESENCE OF POISONOUS AMINES IN THE MAMMALIAN ORGANISM.

By KARL K. KOESSLER and MILTON T. HANKE.

(*From the Otho S. A. Sprague Memorial Institute and Department of Pathology, University of Chicago, Chicago.*)

Amine-producing microorganisms, *i.e.* such microorganisms which decarboxylate histidine to histamine and tyrosine to tyramine, are constant normal inhabitants of the human intestinal tract.

This decarboxylase activity is a specific function of certain strains within the same species; microorganisms which decarboxylate histidine do not decarboxylate tyramine, and *vice versa.*

Decarboxylation of the amino acids, histidine and tyrosine, and probably of other amino acids, is a normal pathway in the catabolism of proteins which occurs side by side with deamination.

Histamine and probably also tyramine, is a normal constituent of the cecal and fecal matter of man; *i.e.*, of the large intestine.

In the detoxication of histamine the liver seems to play only a very subordinate part.

The conclusion is reached, tentatively, on the basis of experimental work, that histamine is rendered pharmacologically inert in its passage through the intestinal wall by the cellular activity of the intestinal mucosa itself.

THE SOLUBILITY OF COAGULATED PROTEINS AS INDICATED BY IMMUNOLOGICAL METHODS.

By H. GIDEON WELLS and JULIAN H. LEWIS.

(*From the Otho S. A. Sprague Memorial Institute and Department of Pathology, University of Chicago, Chicago.*)

Immunological methods furnish a far more delicate means of determining the presence and character of minute quantities of proteins in solution than chemical analytic methods. By means of anaphylaxis and complement fixation reactions a study has been made of the solubility of egg white and horse serum proteins after complete coagulation by heat and removal of uncoagulated proteins by washing. It was found that coagulated egg white suspended in chloroform-water for long periods of time comes occasionally, but not always, to contain barely sufficient dissolved egg protein in 5 cc. samples to sensitize guinea pigs to egg albumin and egg globulin, as well as to egg white. The amount of whole protein in such a solution is probably something less than 0.001 mg. and probably more than one-twentieth of this amount. Coagulated horse serum is somewhat more soluble than coagulated egg white, 5 cc. doses of the supernatant fluid often containing sufficient dissolved antigenic protein to sensitize fatally, probably about 0.001 mg. or slightly more. Complement fixation tests corroborate the anaphylaxis tests and demonstrate that the antigenic protein is actually in solution. Specificity tests indicate that the protein so redissolved is the same protein that was originally coagulated; therefore, the coagulation of protein by heat is to a slight extent a true reversible reaction. Coagulated egg and serum proteins suspended in a finely divided state and injected intraperitoneally into guinea pigs undergo sufficient solution after injection to sensitize the animals to the same uncoagulated protein. Usually the sensitization obtained in this way is greater than when the protein is dissolved in water *in vitro*. Probably the reaction or salt content of the body fluids is more favorable to solution of the coagulated proteins than is pure water, but it is possible that partial enzymatic hydrolysis of the coagulated proteins in the body fluids facilitates solution of the minute amounts of antigen necessary for sensitization.

A PHYSICOCHEMICAL METHOD OF CHARACTERIZING PROTEINS. V.

By EDWIN JOSEPH COHN.

(From the Department of Physical Chemistry in the Laboratories of Physiology, Harvard Medical School, Boston.)

I.

In 1858 Denis described a class of proteins in the blood and in certain seeds that were soluble in salt solutions. This class of proteins, the globulins, has since been found to have a wide distribution, but no satisfactory explanation[1] of their unique relations to electrolytes has thus far been proposed. Such an explanation is now possible as a result of the extensive investigations of Noyes and Harkins and their coworkers, and more recently of Brönsted upon the solubility in salt solutions of compounds of different valence types. Their results indicate that the solubility of slightly soluble compounds is increased by the presence of salts without a common ion and that this solvent action is greater the higher the valence of the saturating compound. We find that the solvent action of salts upon globulins is only slightly greater than that upon the tri-trivalent metal ammonia compounds studied by Brönsted. We therefore conclude that the great solvent action of salts upon globulins depends upon the high valency of these proteins at their isoelectric points.

II.

Recent experiments indicate that salts also increase the solubility of other slightly soluble proteins. The extent to which the solubility of casein is increased by very low concentrations of salt suggests that this protein must be considered either a bivalent, or a uni-trivalent compound.

Brönsted has employed the following equations for the change in solubility from S_0 to S_1, or S_1 to S_2, brought about by an increase in the concentration of total salt:[2]

$$\log \frac{S_1}{S_0} = \alpha\left(c_1^{\beta} - S_0^{\beta}\right) \text{ or } \log \frac{S_2}{S_1} = \alpha\left(c_2^{\beta} - c_1^{\beta}\right)$$

Both α and the exponential β are here constants. This equation is closely related to the empirical law for the lowering of the freezing point proposed by G. N. Lewis: $(1-Y) = \alpha c^{\beta}$. The equations differ chiefly in the last term, employed by Brönsted to designate the stationary state of solubility. The constants have approximately the same value. As a first approximation the simpler form of the equation may be used. Moreover, if the ionic strength, u, be substituted for the concentration, solvents of all valence types may be considered. Taking the logarithms of both sides we have:

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[1] The suggestion of Hardy that globulins form complex compounds with salts, although probably correct, offers no explanation of the unique properties of these proteins, since other proteins also possess the same chemical groups as the globulins.

[2] I am indebted to Professor S. P. L. Sörensen for suggesting to me, in 1920, the use of this equation in describing the effect of salts upon globulins.

$$\log\left(\log\frac{S}{S_0}\right) = \log\alpha + \beta\log u$$

If the left side of this equation be plotted as ordinate, and log u as abscissæ, β is the slope and log α the value of the ordinate, when $\log u = 0$. Extrapolation yields the following constants, which are compared with the constants obtained with Brönsted's equation in Table I.

TABLE I.

Compound.	Type of valence.	Method.	$\log\frac{S_0}{S} = \alpha c^{\beta}$		$\log\frac{S_2}{S_1} = \alpha\left(c_2^{\beta} - c_1^{\beta}\right)$	
			α	β	α	β
NaCl........	Uni-uni.	Freezing point lowering.	(0.33)	(0.54)		
TlCl........	“	Solubility ratio.	0.35	0.52	0.36	0.33
$MgSO_4$.......	Bi-bi.	Freezing point lowering.	(1.44)	(0.38)		
Xantho chromate.......	“	Solubility ratio.	1.0	0.40	1.3	0.2
Casein........	Unknown.	“ “	1.0	0.40		
Luteo hexacyano.	Tri-tri.	“ “	2.0	0.33	3.0	0.2
Serum globulin.........	Unknown.	“ “			3.0	0.2

It should be noted that the values of α, in the Brönsted equation, increase with the square of the valence of the solute in the same way that W. B. Hardy and G. N. Lewis have shown that the ionic strength of the solvent increases with valence. These relations offer a method of investigating and determining the dissociation of such multivalent substances as the proteins.

A PHYSICOCHEMICAL METHOD OF CHARACTERIZING PROTEINS. VI.

By EDWIN JOSEPH COHN.

(From the Department of Physical Chemistry in the Laboratories of Physiology, Harvard Medical School, Boston.)

In the previous communication[3] we have described the increase in solubility of proteins upon the addition of salt by the equation:

$$\log \frac{S}{S_0} = \alpha u^{\beta} \qquad (1)$$

This equation should hold provided solvent and solute have no common ion. In the presence of a common ion, however, the solubility product, or its function scf, should be introduced as the left side of the equation, as Brönsted has shown. The approximation equation then becomes:

$$\log \frac{scf}{s_0c_0f} = \alpha u^{\beta} \qquad (2)$$

When small amounts of salt are added to casein the solubility increases, as we have reported.[3] Upon the addition of larger amounts of salt the solubility decreases. This can be explained if we assume that casein forms a complex compound with the salt, and that both effects of salt are then simultaneously manifest. The effect of all concentrations of salt upon casein can be described by combining equations (1) and (2).

We believe that these conceptions and equations are applicable to other proteins than casein. If proteins that are completely soluble in salt solutions have formed complex compounds, then their precipitation from stronger salt solutions is described by equation (2).

Moreover, the application of this equation to the precipitation of proteins by neutral salts offers a method of characterizing very soluble proteins, for the constant, α, in equation (2), as in equation (1), is a function of the valence of the protein.

[3] Cohn, E. J., *J. Biol. Chem.*, 1924, lix, p. iv.

POSTSCORBUTIC NUTRITION IN THE GUINEA PIG.

By ARTHUR H. SMITH and WILLIAM E. ANDERSON.

(*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.*)

The purpose of the present study was to determine the effect of acute scurvy on the appetite and growth of guinea pigs during the period of recovery. Animals of the same age and of litters of the same size were employed. In each experiment two animals of the same sex were used; an experimental and a control. The diet given throughout consisted of:

	per cent
Soy bean meal (autoclaved)	86
Cod liver oil	5
Dried yeast	3
Calcium lactate	3
Sodium chloride	3

On this food plus 20 gm. of raw cabbage daily normal growth was obtained as determined in preliminary trials comparing the behavior on this diet with that on hay, oats, and cabbage and also with that of Minot's[4] animals. On the soy bean food plus 0.8 gm. of dried cabbage[5] scurvy ensued in from 13 to 16 days with animals of 150 to 200 gm. of body weight. The procedure was so planned that the experimental pig received food *ad libitum* while the control animal received only as much food as the experimental pig ate on the previous day. The effect on the growth rate of inanition *per se* was thus controlled.

The food intake of the experimental animals decreased with the onset of scurvy accompanied by a significant loss in body weight as the disease progressed. The control although suffering the same diminution of food intake as the experimental animal showed far less loss in weight which emphasizes the fact that the development of scurvy is accompanied by a definite loss of body tissue.

After the experimental animals had been given fresh cabbage they immediately resumed growth at the same rate as that of the control animals. A comparison of the food intake of the two groups after the fresh cabbage had been given indicates that as the scorbutic symptoms disappeared and as normal growth was resumed, the experimental animals consumed on the average definitely more food than did the control guinea pigs.

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[4] Minot, C. S. Senescence and rejuvenation, *J. Physiol.*, 1891, xii, 97.

[5] The fresh cabbage was boiled in water for 1 hour and dried in an oven in air at 90–100°C. for 2 days. 0.8 gm. of cabbage thus treated was obtained from 20 gm. of the fresh leaves.

From the above statements of the experimental results it appears that, if after severe, acute scurvy, the young guinea pig survives, subsequent growth proceeds at the normal rate accompanied, however, by a consumption of food definitely greater than that of the control animal under identical conditions.

DETERMINATIONS OF VITAMIN A.

By H. STEENBOCK, MARIANA T. NELSON, and ARCHIE BLACK.

(*From the Department of Agricultural Chemistry, University of Wisconsin, Madison.*)

It has been current practice to determine comparative amounts of vitamin A in different foodstuffs by taking observations on the growth of rats when abundantly supplied with good protein, salts, roughage, vitamin B, and energy. From analysis of previous data comparing the incidence of ophthalmia with time of cessation of growth, it appears that a lack of the antirachitic vitamin in the absence of vitamin A may likewise be responsible for growth inhibition. This throws in doubt the value of many data of vitamin A distribution where growth failure has been used as a sole criterion of its absence. Experimentally advantage has been taken of the fact that apparently ultra-violet light has the property of substituting for the antirachitic vitamin. By its means it has been found that in many cases of so called vitamin A deficiency, growth could be reinitiated by radiation and maintained until incidence of an ophthalmia. This is taken to indicate primarily a deficiency of antirachitic vitamin rather than one of vitamin A. Using this technique a distribution of vitamin A has been studied in a number of grains and forage plants. It has been found that some millets are poor in the antirachitic vitamin but comparatively well supplied with vitamin A. The same has been found true of clover and alfalfa. Bone analyses were used for control purposes throughout.

THE USE OF THE RAT FOR THE ESTIMATION OF VITAMIN B.

By M. H. GIVENS and F. BEHRENDT.

(From the Research Laboratories, Northwestern Yeast Company, Chicago.)

From experiments in which old and young rats were fed and caged differently evidence has been obtained that:

The rat does not store in its body a large excess of vitamin B. This result is in accord with the findings of Steenbock and associates.[6]

The rat can supplement only slightly its intake of vitamin B by ingesting its feces. Steenbock, Sell, and Nelson[7] say: "When rats are prevented from supplementing their diet by the consumption of excreta the vitamine B content of the experimental rations must be at least twice as high."

There does not appear to be a definite relation between the age of a rat and its duration of life on a diet devoid of vitamin B but otherwise satisfactory.

In the absence of vitamin B in its diet, the duration of life of a rat appears to depend mainly on the animal's continuously consuming this ration in an amount approaching its maintenance requirement. Initial weight seems to be secondary to this factor.

Young rats declining on a diet free of vitamin B have recovered and grown for over 300 days on a daily supplement of 50 mg. of dried yeast. These experiments show quite clearly the relation of growth to food consumption.

Litter mates, though treated exactly the same in every respect, do not always grow alike because they do not always eat alike. The rat is the one variable in feeding experiments which cannot be controlled.

The foregoing results have a direct bearing on the use of the rat for the estimation of vitamin B.

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[6] Steenbock, H., Sell, M. T., and Jones, J. H., *J. Biol. Chem.*, 1923, lv, 411.

[7] Steenbock, H., Sell, M. T., and Nelson, E. M., *J. Biol. Chem.*, 1923, lv, 399.

QUANTITATIVE ASPECTS OF THE RELATION BETWEEN VITAMIN B AND APPETITE.

By GEORGE R. COWGILL, H. J. DEUEL, Jr., and ARTHUR H. SMITH.

(From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.)

A method of feeding dogs on diets consisting of isolated food substances and adequate except with respect to vitamin B was employed.[8] Under these conditions the animals sooner or later lose all desire to eat; when a sufficient amount of some source of vitamin B is then administered, the appetite is quickly and completely restored.

We have experimented with two sources of vitamin B, namely the *Yeast Vitamin Powder (Harris)*[9] and *Wheat Sugar*[10] made from wheat embryo. Preliminary experiments in which the number of days over which a single dose of the yeast preparation was effective showed the dog's requirement of this material to be between 50 and 60 mg. per kilo of body weight per day. Long continued feeding experiments were then performed in which the Harris powder was weighed out, placed in gelatin capsules, and a capsule administered to the animal each day. 20, 30, 40, 50, and 60 mg. per kilo per day were thus tested. 20 mg. proved distinctly inadequate; the 30 mg. dose was associated with maintenance of appetite over 91 days in one case and 59 days in another. The 40, 50, and 60 mg. doses gave successful results for as long as the animals were kept on the experiment, in every case, at least 3 months. On December 5, 1923, the two animals receiving 40 mg. per kilo per day had eaten the same diet perfectly for 352 days—almost a year. The minimum vitamin B requirement of the dog when fed under these conditions, appears, in terms of the Harris powder, therefore, to be between 30 and 40 mg. per kilo of body weight per day. The accuracy of this method is not great enough to warrant a more precise statement in this regard.

The *Wheat Sugar*, after failures with smaller amounts, gave successful results in two cases over periods of approximately 7 and 8 months, respectively, with 0.6 gm. per kilo per day. Administration of this product was then stopped; later the animals lost their desire for food, a condition which was promptly cured by the renewed administration of this vitamin-containing product. This quite apart from its relation to the quantitative aspects of this problem, would seem to be a complete demonstration of the relation between vitamin B and appetite. When these two products are compared as to their content of vitamin B by the growing rat method, the same ratio of potencies is obtained.

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[8] Cowgill, G. R., *J. Biol. Chem.*, 1923, lvi, 725.

[9] From the Harris Laboratories, Tuckahoe, N. Y.

[10] From the Ward Baking Co., New York, N. Y.

THE INFLUENCE OF YEAST PRODUCT ADDITIONS TO MILK RATIONS ON THE INFERTILITY OF RATS.

By H. A. MATTILL and C. C. CONGDON.

(From the Department of Physiology, University of Rochester, Rochester.)

The apparent improvement in reproductive function of female albino rats when yeast is added to milk rations has been found not to be due to yeast nucleic acid or to vitamin B. When hot water extracts of yeast are treated with adsorbents such as Lloyd's reagent or charcoal and the filtrate (containing Funk's vitamin D) is evaporated on starch and incorporated into milk rations to the extent of 5 or 10 per cent of the original yeast there is some improvement. This change is more marked in the case of charcoal (norit) filtrate than it is with the filtrate from Lloyd's reagent. Of eleven animals on the latter product eight showed one or more resorptions, but no births. Ovulation was regular. Of eleven animals on the norit product seven, while showing some resorptions also gave birth to ten litters, only one of which lived, two animals showed neither births nor resorptions. It would appear, therefore, since our animals on milk rations seldom, if ever, show even as much as resorption, that the charcoal filtrate provides something in the nature of Evans' vitamin X. The failure of the Lloyd's reagent filtrate can be explained by the better adsorbing qualities of this substance. Preliminary experiments on the addition of vitamin X in the form of lettuce, to milk-fed rats, indicate that this unknown is indeed one of the inadequacies of milk; but the mortality of the young is high, thus suggesting that the X factor is not the sole addition necessary to make a milk ration adequate. In male animals the variability of response to the yeast products is much higher than in the females. In a very few cases the early degeneration of the testes so constantly found in milk-fed animals is delayed. This degeneration has been postponed but not prevented by addition of green lettuce to milk rations. Work on wheat embryo and preparations from it is in progress.

THE AVAILABILITY OF SOME POSSIBLE PRECURSORS OF LYSINE FOR LYSINE SYNTHESIS.

By HOWARD B. LEWIS, DANIEL A. McGINTY, and CARL S. MARVEL.

(From the Laboratories of Physiological Chemistry, University of Michigan, Ann Arbor, and of Organic Chemistry, University of Illinois, Urbana.)

In a previous study,[11] we have demonstrated that it is not possible to utilize nor-leucine in place of lysine to supply the chemical factor necessary for normal growth on diets otherwise complete but deficient in their lysine content. Similar experiments have been made with various other possible precursors, α-hydroxycaproic acid, ϵ-aminocaproic acid, and α-hydroxy-ϵ-aminocaproic acid. Young white rats were maintained on a diet in which the protein element was supplied by gliadin as follows: gliadin, 18 per cent; salt mixture (Osborne-Mendel), 4.5 per cent; starch, 50 per cent; lard, 24.5 per cent; cod liver oil, 3 per cent. Water-soluble vitamin was supplied in the form of yeast concentrate (Yeast Vitamine-Harris). With this type of diet, maintenance or very slow growth only was possible. Substitution of casein for gliadin in these diets resulted in a normal rate of growth. When any one of the caproic acid derivatives above mentioned was added to the gliadin diet, no change in the rate of growth was noted, although addition of lysine itself to the gliadin diet caused a marked increase in weight and a normal rate of growth. The failure of α-hydroxy-ϵ-aminocaproic acid to act as an available precursor of lysine is of particular interest, since it apparently demonstrates that the amination of α-hydroxy acids, observed in perfusion experiments, is not possible in the living organism, at least under the conditions of the present series of experiments.

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[11] Lewis, H. B., and Root, L. E., *J. Biol. Chem.*, 1920, xliii, 79.

THE RELATION OF ARGININE AND HISTIDINE TO GROWTH.

By WILLIAM C. ROSE AND GERALD J. COX.

(*From the Laboratory of Physiological Chemistry, University of Illinois, Urbana.*)

Comparative studies have been made of the growth of rats upon diets in which the nitrogen was supplied, respectively, by casein, completely hydrolyzed casein, and hydrolyzed casein from which arginine and histidine had been removed by the usual method of silver precipitation. Rats upon completely hydrolyzed casein grew to maturity, but at slower rates than animals of the same age upon whole casein. Rats upon the arginine-histidine-free amino acid mixture were neither able to grow nor to maintain body weight, but promptly and continually lost weight. The addition of histidine to such a ration resulted invariably in an immediate resumption of growth, at a rate almost or quite equal to that of animals upon normal diets.

Experiments designed to determine the minimum histidine requirement showed that the addition of 0.1 gm. of histidine monochloride per 100 gm. of food (corresponding to approximately 0.5 per cent of the protein when the monochloride is expressed as free histidine) usually sufficed for *maintenance.* 0.2 to 0.3 gm. of histidine monochloride per 100 cm. of food permitted *moderate growth,* while 0.5 gm. (equivalent to 2.5 per cent of the protein, the proportion of histidine normally present in casein) occasioned *a practically normal rate of increase in body weight.* The addition of histidine to the ration was followed in every case by a pronounced increase in food consumption. This occurred whether the amino acid was mixed with the diet, or was fed separately.

In contrast to the behavior of histidine, the addition of arginine to the deficient diet exerted no perceptible influence upon growth, even when the quantity added was more than equivalent to the sum of the arginine and histidine present in native casein. The animals continued to lose weight as rapidly as before the addition of the amino acid. Nor could growth be induced by arginine in animals upon the minimum maintenance allowance of 0.1 per cent of histidine monochloride. We are therefore forced to the conclusion that, contrary to the observations of Ackroyd and Hopkins,[12] *arginine and histidine are not mutually interchangeable in metabolism.* The experiments have no bearing upon the question of the indispensability of arginine, since it is quite likely that the Kossel-Kutscher method of precipitation does not remove arginine as completely as it does histidine.

Attempts to satisfy the histidine requirement by the inclusion in the diet of creatine or creatinine gave negative results. Despite the fact that creatinine contains the glycocyamidine ring, closely related to the imidazoles in structure, and that creatine is readily transformed into creatinine, neither substance was able to replace histidine.

The investigation is being continued and extended to other imidazole derivatives. We are also determining the influence of the diamino acids upon the tissue concentration and urinary excretion of purines, allantoin, creatine, and creatinine.

AMINO ACIDS IN NUTRITION.

VIII. THE INDISPENSABILITY OF PROLINE FOR GROWTH.

By BARNETT SURE.

(*From the Department of Agricultural Chemistry, Agricultural Experiment Station, University of Arkansas, Fayetteville.*)

After more than 50 experiments involving some 200 animals for the study of the rôle of proline in nutrition had been employed, two experiments were finally struck which clearly show the limited synthetic capacity of the animal organism towards the pyrrolidine nucleus of the protein molecule; namely, proline. Since edestin was found to be deficient in cystine and lysine, and since gelatin, although it is deficient in cystine, tyrosine, and tryptophane, was found to supplement edestin very remarkably in the presence of cystine,[13] experiments were initiated for the purpose of finding out the possible supplementary nature of gelatin, employing edestin as the protein for this investigation and keeping in mind that gelatin is abundant in proline and arginine. Four sets of experiments were started, employing edestin at 9 and 6 per cent levels of protein intake; the control experiments containing 0.4 per cent cystine and 0.4 per cent lysine, and the other rations 0.4 per cent proline in addition. All the rations were fortified with an abundance of all the other dietary factors, so that proline was the only variable constituent. On the 9 per cent edestin rations no particular advantage was derived by the addition of proline, but the response to proline was very striking on the 6 per cent edestin levels. The experiments were conducted with young albino rats in groups of four, beginning at weaning time, and were carried on for 20 weeks. Accurate food consumption records were kept throughout the period of experimentation. Two animals on the 6 per cent edestin-cystine-lysine ration made very poor growth, while two animals on the 6 per cent edestine-cystine-lysine-proline diet made practically normal growth. For 3 months it was not apparent whether two animals on the proline ration were doing much better than the control experiment. At that point arginine to the extent of 0.4 per cent of the total ration (as a common amino acid) was added to the rations of these groups of animals, which were confined in individual cages, and quantitative records kept of their individual food consumption. Calculating the gain in weight per gram of protein intake, it became very evident that the response to proline in the presence of arginine was very remarkable. Responses to proline were then secured on 6 per cent edestin rations in the presence of cystine and lysine in some animals, and in the presence of cystine, lysine, and arginine in others.

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[12] Ackroyd, H., and Hopkins, F. G., *Biochem. J.*, 1916, x, 551.

[13] Sure, B., *Am. J. Physiol.*, 1922, lxi, 1.

Proline is considered by the author as an essential amino acid for growth.

AMINO ACIDS IN NUTRITION.

IX. THE RÔLE OF ALANINE AND INDOLE IN THE SYNTHESIS OF TRYPTOPHANE BY THE ANIMAL ORGANISM.

By BARNETT SURE.

(From the Department of Agricultural Chemistry, Agricultural Experiment Station, University of Arkansas, Fayetteville.)

Since tryptophane was found to be the determining growth-limiting factor in the proteins of corn, an attempt was made to find whether tryptophane, which is indole-alanine, could be synthesized by the animal organism when indole and alanine (introduced in the form of the leucine fraction from a hydrolysis of zein) are incorporated in the diet. The results of the experiments show that the albino rat is unable to perform such a synthesis when the two components of the tryptophane molecule are administered orally.

ON THE ABSORPTION OF ORGANIC COLLOIDS BY THE INTESTINAL MUCOSA.

By A. B. MACALLUM.

(*From the Department of Biochemistry, McGill University, Montreal, Canada.*)

Except in the case of fats and soaps, little has been determined regarding the mode of absorption of organic colloids by the epithelial cells of the intestinal mucosa and the manner of transfer through the cells to the interior of the villi. This is due to the fact that there are no microchemical stains which will localize the absorbed proteins as the fats and soaps are revealed by dyes (Sudan III and scarlet red) and osmic acid in the cells.

Some comprehension of the forces involved in this absorption may be obtained from a study of the results of feeding guinea pigs and rabbits, the former more especially, with fresh undiluted egg yolk for a day or more, after they have been kept without food for 24 to 48 hours. The yolk is, in a fluid condition, introduced into the esophagus by a pipette and as much as 10 cc. may thus be given three times a day. Thus given, and in such amounts, the yolk, practically unaffected by gastric digestion, reaches the intestine and immediately comes in contact with the tips of the villi, the cells of which begin at once to take it up and transfer it to the underlying tissues, but as the latter do not absorb it as fast as the cells deliver it to them an accumulation of it occurs at their bases which at the end of 24 hours is so great that the epithelial layer at the tips is "ballooned" or raised to a height several times the long diameter of the normal cells above the "basement membrane," and the cells are flattened by the pressure to which they are subjected internally. They continue to absorb it and at the end of 48 hours or more the "balloons" at the tips of all the villi are ruptured and the contents escape into the intestinal cavity, but before this rupture occurs some of the absorbed material is transferred through the adenoid tissue to the lacteals which are in consequence greatly distended and their contents contain some at least of the proteins as well as the fats of the absorbed yolk.

A careful examination of the epithelial cells, both in the fresh and in the fixed condition, during the early stages of this absorption makes it evident that in this absorption the cells are active, not passive elements. The contents of the yolk spherules, which are all distintegrated, come into intimate contact with the free borders of the cells, the proteins dissolve in the protoplasmic processes which also take up the fat particles which are almost of ultramicroscopic size and both proteins and fats are transferred through the pores of the basement membrane to the interiors of each cell. Here the cytoplasm dissolves the proteins or enters into intimate relations with them and they diffuse towards the base of the cell where they are set free in a concentrated form with such amounts of the lipoids as are transferred there in a micellar condition. In this passage through the cell the proteins may lag behind the lipoids and as a result one may find in certain of the cells, particularly in those at the sides of the tips of the villi, spherules of protein, of 3 to 4 μ in diameter, free from lipoids, lying in cavities of the cytoplasm which is, however, setting free proteins and lipoids at the basal end of the cell.

The "ballooning" of the epithelial covering of the villi and the great distention of the lacteals indicate that in the transfer of the proteins and the lipoids to the lacteals, the epithelial cells and the cells of the underlying adenoid tissue, work against a pressure which cannot be classified as osmotic pressure as generally understood. Probably the force known as intrinsic pressure plays a very important part in this transfer.

The interpretation of these results has a bearing on the transfer of proteins and lipoids from the blood to the tissues and on the diffusion of these through and from the cells of the tissues.

DIGESTIBILITY OF PROTEINS AND THE ISOELECTRIC POINT.

By A. B. HERTZMAN and H. C. BRADLEY.

(From the Department of Physiological Chemistry, University of Wisconsin, Madison.)

Foreign proteins, such as albumins and globulins, digest in autolyzing liver brei at characteristic pH values. These values correspond closely to the isoelectric points of these proteins. This indicates that the native protein must be converted into its acid salt or the ion of such a salt before it is substratum. Thus edestin is found to digest at pH 7.0; its isoelectric point is 6.9 (Rona and Michaelis). Egg albumin digests at a pH of 5.0; its isoelectric point is 4.8 (Sörensen).

Egg or serum albumins and globulins inhibit autolysis in a pH range where liver itself digests very well. The inhibition is not complete even with large additions of the foreign protein: The inhibition does not affect the final equilibrium attained but does decrease the speed of the reaction and in proportion to the amount of foreign protein added. The effect of the foreign protein is, therefore, to diminish the active mass of catalyst present without altering the mass of substratum in any way. This indicates the formation of a stable protein-enzyme combination which does not proceed to cleavage, but eliminates a fraction of the enzyme from the reaction.

THE MECHANISM OF THE ACTION OF AMINO ACID PROMOTERS UPON ENZYMES.

By E. W. ROCKWOOD.

(From the Department of Chemistry, the State University of Iowa, Iowa City.)

The activity of urease and ptyalin in solution has been measured, alone and in the presence of α-amino acids which act as promoters. The rate of decay of these enzymes has also been measured. The mechanism of the action of the promoters has been studied and found to consist of two factors; one is the prevention of the decay of the enzyme, but the greater part of the action of the promoter is not due to this. There is a specific stimulation of the enzyme by the amino acid. Possible hydrogen ion effects were eliminated by the use of a buffer.

UREASE AND THE JACK BEAN PROTEINS.

By J. B. SUMNER, V. A. GRAHAM, and C. Y. NOBACK.

(From the Department of Biochemistry, Cornell University Medical College, Ithaca.)

The jack bean has been shown by one of the authors to contain two crystallizable globulins, a non-crystallizable globulin[14] and a phosphatide, which is a powerful thrombokinase.[15] In addition there is present a pentose gum and a colloidal yellow pigment. Albumin is absent.

It was thought of interest to see if any of the above substances were concerned in any way with the activity of urease.

Methods of extraction, precipitation, and dialysis combined with methods for the differential adsorption of both proteins and urease have led to the preparation of urease solutions free from both carbohydrate and protein.

The properties of the purified enzyme are discussed.

THE ACID PROPERTIES OF HEMOGLOBIN.

By A. B. HASTINGS, D.D. VAN SLYKE, J. M. NEILL, and M. HEIDELBERGER.

(From the Hospital of The Rockefeller Institute for Medical Research, New York.)

At constant pH oxygenated hemoglobin binds more alkali than reduced hemoglobin. Quantitative measurements have been performed on solutions of recrystallized horse hemoglobin in order to determine the magnitude of the effect at varying reactions. The increase in base bound per molecule of oxygen combined has been found to depend on this reaction. A maximum value of 0.7 equivalent of base added per mol of oxygen has been observed at pH 7.6. At lower pH the value diminishes, falling to 0.4 at pH 6.8. The curve expressing the observed variations of the value with pH agrees with that calculated according to L. J. Henderson's hypothesis, that oxygenation and reduction alter the dissociation constant of one acid hydrogen in the hemoglobin molecule. The observed values agree with those calculated on the assumption that $K_O = 10^{-6.87}$, $K_R = 10^{-8.33}$, where K_O and K_R represent the dissociation constants of the one labile acid group in the oxygenated and reduced hemoglobin, respectively.

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[14] Sumner, J. B. The globulins of the jack bean, *Canavalia ensiformis*, *J. Biol. Chem.*, 1919, xxxvii, 137.

[15] Sumner, J. B. Sur le cytozyme retiré des graines de canavalia ensiformis, *Compt. rend. Soc. biol.*, 1922, lxxxvi, 108.

The relationships between base-binding power, reaction, and degree of oxygenation of hemoglobin are expressed by the equation

$$\underbrace{[\mathrm{BHb}]}_{\substack{\text{Base bound}\\ \text{by total}\\ \text{hemo-}\\ \text{globin.}}} = \underbrace{\beta_R\,[\mathrm{Hb}]\,(\mathrm{pH} - \mathrm{I}_R)}_{\substack{\text{Base bound}\\ \text{by hemoglobin}\\ \text{in the re-}\\ \text{duced state.}}} + \underbrace{[\mathrm{HbO_2}]\left(\frac{1}{1 + 10^{\mathrm{pK}'_0 - \mathrm{pH}}} - \frac{1}{1 + 10^{\mathrm{pK}'_R - \mathrm{pH}}}\right)}_{\substack{\text{Additional base bound as the result}\\ \text{of oxygenation.}}}$$

$[\mathrm{Hb}]$ = total hemoglobin, $[\mathrm{HbO_2}]$ = oxygenated portion, β_R = molecular buffer value of reduced hemoglobin, I_R = isoelectric point of reduced hemoglobin, $\mathrm{pK}'_O = -\log \mathrm{K}'_O$, $\mathrm{pK}'_R = -\log \mathrm{K}'_R$.

STUDIES ON OXIDATION-REDUCTION EQUILIBRIA IN SYSTEMS OF ORGANIC COMPOUNDS.

By W. MANSFIELD CLARK, BARNETT COHEN, H. D. GIBBS, and M. X. SULLIVAN.

(*From the Division of Chemistry, Hygienic Laboratory, United States Public Health Service, Washington.*)

Electrometric measurements of the oxidation-reduction potentials of bacterial cultures had given highly interesting data but reliability appeared uncertain and, accordingly, a study has been made of indicators which furnish material for a theoretical study of organic systems and a semi independent method of checking electrode data.

A variety of indicators has been studied in a preliminary way and detailed data have been obtained for the sulphonates of indigo and a variety of indophenols. Electrode potentials are a function of the ratio of reductant to oxidant and of the hydrion concentration of the solution. Equations confirmed by experiment express the influence of the several acid or basic dissociation constants. These as well as the so called normal potentials have been determined and reveal in quantitative terms the effects of chemical substitution.

With the data obtained certain zones in the scale of oxidation-reduction potential have been covered by a system of indicators which should be useful in physiological studies.

ALKALOSIS IN DOGS FOLLOWING INJECTION OF HYDRAZINE SULFATE.

By B. M. HENDRIX and AVA J. McAMIS.

(From the Laboratory of Biological Chemistry, School of Medicine, and the Laboratory of Chemistry, College of Pharmacy, University of Texas, Galveston.)

It has been shown that a decrease in blood glucose is produced in the dog by injection of hydrazine sulfate. This substance is known to produce liver injury.

The work of Sweet and one of us shows that the carbon dioxide-combining power of the blood may be higher than normal in Eck's fistula diabetic dogs. We have supposed that liver injury brought about this alkalosis.

The object of this investigation is: (1). To see if alkalosis follows the liver injury produced by hydrazine sulfate. (2). To see if there is any relation between the change in pH and the alkali reserve, and the appearance of hypoglycemia.

We have found that injections of hydrazine sulfate increase both the pH and the carbon dioxide-combining power of the blood of dogs. This happens not only when one relatively large injection is given, but also when small doses are given at 48 hour intervals. The hypoglycemia develops to a certain extent at least, in either case.

In a general way, the most pronounced hypoglycemia occurs either on the day of greatest alkalosis or on the day following.

SOME VARIATIONS IN THE ACID-BASE BALANCE OF THE BLOOD IN DISEASE.

BY VICTOR C. MYERS AND LELA E. BOOHER.

(From the Department of Biochemistry, New York Post-Graduate Medical School and Hospital, New York.)

The explanation of the normal and abnormal variations in the acid-base balance of the blood, advanced by Van Slyke,[16] is simple but comprehensive, and has been of very great assistance to us in the study of clinical conditions of acidosis and alkalosis.

As a measure of the acid-base balance we have estimated the CO_2 content and pH of the blood plasma, the former by the method of Van Slyke and the latter by a modification of the Cullen colorimetric technique previously described by us.[17] About 200 specimens of blood have been examined and among these we have encountered conditions falling into each of the nine areas described by Van Slyke. In our experience the pH of normal individuals falls between 7.35 and 7.43, and we are inclined to regard figures below 7.32 and above 7.47 as definitely abnormal. Our series of cases has included eight cases of uncompensated alkalosis and sixteen cases of uncompensated acidosis, the highest pH observed being 7.60 and the lowest 6.98. The eight cases of alkalosis all received sodium bicarbonate therapy; although in three instances the amount given was very small and was probably not the most important factor involved in the alkalosis. Two cases were receiving Sippy treatment. One case had received radium therapy. Vomiting occurred at irregular intervals in five of the cases, and in at least one case was the probable cause of the alkalosis. Several cases also showed a slight rise in temperature. In the eight cases the plasma pH ranged from 7.49 to 7.60 and the CO_2 content from 56 to 83. Alkalosis is not necessarily associated with an abnormally high blood bicarbonate and it seems evident that with continued bicarbonate therapy the CO_2 content may be reduced to a normal value while the pH increases to an abnormal figure.

Of the sixteen cases of uncompensated acidosis, five were diabetic and eleven were suffering from renal insufficiency. The pH values ranged from 7.32 to 6.98 and the CO_2 content from 3.8 to 57. Two severe nephritics lived for nearly a month in a condition of uncompensated acidosis.

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[16] Van Slyke, D. D., *J. Biol. Chem.*, 1921, xlviii, 153.

[17] Myers, V. C., Schmitz, H. W., and Booher, L. E., *J. Biol. Chem.*, 1923, lvii, 209.

THE FORMS OF COMBINATION OF THE UNSATURATED FATTY ACID IN BLOOD PLASMA.

By W. R. BLOOR.

(From the Department of Biochemistry, Medical School, University of Rochester, Rochester.)

In earlier work it was shown that fatty acids of a relatively high degree of unsaturation were to be found in blood plasma. Since these substances are probably of importance as stages in the intermediary metabolism of the fats and since in blood plasma they are presumably in process of transportation it was desirable to find out the types of compound in which they occurred. The work presented at this time indicates that most of these acids are in combination with cholesterol with a considerably smaller proportion in combination as lecithin (phospholipoid) and still less as fat. The importance of cholesterol in the intermediary metabolism of the fatty acids is thus indicated.

PLASMA PROTEIN AS AN INDEX OF HYDROPLASMIA DURING PREGNANCY.

By E. D. PLASS and L. JEAN BOGERT.

(From the Department of Obstetrics, Henry Ford Hospital, Detroit.)

The protein content of oxalated plasma from normal nonpregnant young women usually ranges from 6.5 to 7.5 per cent (average 7.00 per cent). No noticeable change occurs during the first 2 months of pregnancy, but beginning with the 3rd month there is a gradual diminution of plasma protein which reaches a maximum at the 5th month. During the last few months of pregnancy the dilution tends to be slightly less marked, and there is a further concentration at the time of labor. During the first 2 or 3 days of the puerperium a redilution occurs, followed by a rapid return to normal, which is attained about the end of the 1st week.

In the late toxemias of pregnancy (eclampsia and its allied conditions), the degree of hydroplasmia is generally exaggerated but tends to follow the same curve.

Hematocrit determinations made at the same time as the plasma protein estimations indicate that the relative plasma volume follows inversely the plasma protein concentration, thereby suggesting that the diminution of the latter is to be looked upon as a true dilution phenomenon. Alterations in the concentration of any serum or plasma constituent during pregnancy may therefore be attributable to variations in blood dilution.

VARIATIONS IN THE BLOOD CHOLESTEROL AND PHOSPHATIDES OF RABBITS DUE TO PREGNANCY.

By EMIL J. BAUMANN and O. M. HOLLY.

(From the Laboratory Division, Montefiore Hospital, New York.)

The increased cholesterol content of human blood during the latter part of pregnancy has been confirmed from so many sources that it may be taken as an established fact, but its significance is still not well understood. Recently it has been observed that the blood phosphatides are also increased in pregnant women.

In pregnant rabbits, the quantities of these substances in the blood show quite different variations from those occurring in human pregnancies. No rise in either the blood phosphatide or cholesterol values is observed; on the contrary, a marked decrease in both occurs, averaging about 40 per cent in the seven animals studied. The decrease began 15 to 20 days after conception and reached the lowest level 1 to 4 days before parturition, (which usually occurs on the 30th day). The cholesterol and phosphatide values rise again, irrespective of lactation, to the non-pregnant figures, 4 weeks and 1 week after parturition, respectively.

In four pregnant thyroidectomized animals, similar but more marked decreases were observed, averaging 60 per cent of the non-pregnant values. This occurs in spite of the fact that thyroidectomy causes a decided increase especially in the amount of cholesterol and to a lesser extent, of the phosphatides of the blood. The return to the non-pregnant values occurs in a shorter time in thyroidectomized animals than in normal animals—usually within 1 week.

In a few animals we have found that the cholesterol and phosphatides of fetal blood are perhaps slightly higher (15 per cent) than in maternal blood; certainly not lower, as has been found in man.

The decreased cholesterol and phosphatide contents of the blood may be regarded as due to a withdrawal of these substances from the mother to the fetus, to meet the requirements for the very rapid growth of the embryos that occurs in the last 2 weeks of pregnancy.

FURTHER OBSERVATIONS ON CHEMICAL CONSTITUENTS OF SALIVA.

By J. LUCIEN MORRIS and CHARLES T. WAY.

(From the Department of Biochemistry, Western Reserve University School of Medicine, Cleveland.)

Supplementing earlier studies of normal salivary secretion, observations were made on pathological cases. Subjects were chosen which exhibited abnormally high concentration in the blood of substances analyzed in saliva. Special attention was paid to urea and uric acid in nitrogen retention cases, such as chronic nephritis, mercuric chloride poisoning, lysol poisoning, and to glucose in diabetes. The amount of blood urea ranged up to more than ten times normal, glucose as high as five times normal, and uric acid to a maximum of somewhat over twice normal.

Glucose results can be briefly summarized: No matter how high the blood sugar concentration in the cases observed, the amount of reducing substance present in saliva was never more than a trace, nor was that trace more than was present in the saliva of subjects having normal blood sugar.

Salivary urea tended to increase with blood urea. However, in terminal nephritis the amount of urea eliminated through the salivary glands did not continue to increase and in some cases was actually less than normal. This was apparently due to failure of the glands to function, possibly because of general failure of body function. In anuria following lysol poisoning, salivary urea percentage exceeded the blood percentage.

Pathological cases emphasized the difference between uric acid and urea secreted in response to various stimuli. In a few cases, salivary uric acid was definitely concentrated beyond the urea content. In most pathological cases studied the uric acid was diminished markedly, often even to the point of disappearance. Administration of pilocarpine was followed in every case by more marked increase in salivary uric acid than in urea.. Such stimuli as chewing caused negligible increases in uric acid, even though volume and urea were usually much increased. This observation confirmed the earlier conclusion that uric acid, more than any other constituent, represents metabolic cellular activity as contrasted with the filtration mechanism.

CHEMICAL CHANGES PRODUCED IN THE BLOOD BY FASTING AND SUBSEQUENT REFEEDING.

By SERGIUS MORGULIS and A. C. EDWARDS.

(From the Department of Biochemistry, College of Medicine, University of Nebraska, Omaha.)

The non-protein and urea nitrogen of the blood of fasting dogs usually increases during the early stages of the inanition and then remains at a more or less fixed level until the extreme stage is reached, when a new and much greater increase in nitrogen occurs. The amino acid nitrogen either remains constant all through the duration of the fast or it may diminish slightly at first to rise once more during the latter part of the fast. The blood uric acid increases progressively during inanition. The creatinine remains constant, but the creatine, following a diminution which may occur at an early stage of the fast, rises rapidly in the ultimate stage. The blood sugar and chlorides which usually diminish in the course of the first half of the fasting period increase again during the later stages of inanition. At the time the body weight of the dogs has lost about 40 per cent or more the blood sugar level may even exceed the normal. With but one exception, the undetermined nitrogen and the percentage of total solids in the blood of our dogs increased during inanition.

On repeating a fast, the blood changes become less pronounced or even fail altogether to appear. The total solids, however, increase as they also did during the first fast.

Fasting without water produces the same effects as fasting with a free supply of drinking water, but the changes occur more quickly and are more pronounced when both food and water are being withheld.

Upon feeding the dogs after a protracted fast extensive changes occur in the blood. The non-protein and urea nitrogen, the uric acid, and creatine decrease rapidly in the first few days of realimentation. The decrease is particularly striking in the non-protein and in the urea nitrogen moieties. As the refed animals, however, have gained 35 to 45 per cent in weight the urea and total non-protein nitrogen begin to increase again tending to return to the original level when the prefasting body weight is about restored. The amino acid nitrogen undergoes slight changes, while the creatinine content of the blood is entirely unaffected by the refeeding. During realimentation the total solids of the blood also fall abruptly. In general the composition of the blood approaches the normal condition as the original body weight of the previously fasted dogs is restored.

THE GLUCOSE EQUIVALENT OF INSULIN ON DEPANCREATIZED DOGS.

By F. N. ALLAN.

(From the Physiological Laboratory, University of Toronto, Toronto, Canada.)

Completely depancreatized dogs were kept on a daily ration of 500 or 600 gm. of meat and 100 gm. of cane-sugar. They were also injected daily with insulin. The glucose balance was compared for periods in which varying quantities of insulin were given and the glucose equivalent of the latter calculated by dividing the change in the glucose balance by the change in the number of units of insulin administered. It was found to vary with the doses of insulin, becoming progressively smaller as this was increased. By plotting glucose equivalents against units of insulin a curve was obtained which may be used for determining the glucose equivalent of an unknown sample of insulin. The falling off in the glucose equivalent with increase in unitage is not mainly due to excretion of insulin at higher dosages since injection of constant amounts of insulin daily with varying amounts of carbohydrate give equivalents which increase as the carbohydrate is increased.

INSULIN FROM FISH.

By N. A. McCORMICK and E. C. NOBLE.

(From the Physiological Laboratory, University of Toronto, Toronto, Canada.)

Continuing the work on the source of insulin from fish, it has been found that easily removable principal islets exist in many other common bony fishes besides *Lophius* and *Myoxocephalus* and that in the cod (*Gadus callarius*) and the halibut (*Hippoglossus hippoglossus*) large islets occur in close relationship to the gall bladder. These have been removed in large quantities of fish and extracts made by a process which consisted in repeatedly extracting the pulpified islets with about 70 per cent alcohol, containing about 0.3 mineral acid. Alcohol was removed from the extract either by evaporation *in vacuo* or by a current of air, the fat removed by means of ether and the fat-free solution then placed in a boiling water bath. The resulting solution can be used with perfect safety for clinical purposes, but it is desirable to purify the insulin further by applying Dudley's picric acid process and then decomposing the picrate with hydrochloric acid. By these methods large yields of insulin have been obtained at relatively small cost from large quantities of fish. Expressing the yield in terms of clinical units (a clinical unit being one-third the amount of insulin required to lower the blood sugar of a normal fasted rabbit to convulsive level in 4 hours) the following number of units per gm. of principal islets have been obtained on different occasions—*cod* 22.7, 11.3, 12.7, 13.6, 20.2, 14.7, 19.3, and 17.0. The variability in these yields is due to deliberate alteration of the temperature and method of preservation of the islets before delivery at the laboratory. In one case the material was kept for 17 days at room temperature before being worked up and the yield was only 5.8 units. The yield from the principal islets of pollack in one case was 35 units per gm. of islets; from halibut 18 units; from hake 15 units; from haddock 28.3 units.

It was also observed in several of these fish that extracts of liver prepared by the above method had a marked hyperglycemic effect. Extracts of zymogenous tissue had practically no effect, but if any, they produced a slight lowering (possibly because of the islets in them).

THE EFFECT OF INSULIN ON THE OXYGEN SATURATION OF HEMOGLOBIN.

By J. M. D. OLMSTED and A. C. TAYLOR.

(From the Department of Physiology, University of Toronto, Toronto, Canada.)

Having noticed that arterial blood in rabbits and cats during the early stages of an insulin convulsion is venous in character, the percentage saturation of hemoglobin with oxygen was determined in decerebrated cats, at half hour intervals after injection of insulin. At the same time variations in the respiratory exchange were recorded. The hemoglobin saturation remained constant for 3 hours until an hour before the appearance of convulsions when it fell from 88 to 80 per cent. At approximately the same time the ventilation of the lungs and the volume of expired CO_2 were both slightly increased, while the volume of O_2 consumed was diminished, causing the R.Q. to rise abruptly to a value slightly above 1. At the beginning of convulsions the oxyhemoglobin had fallen to 75 per cent; ventilation, expired CO_2, and O_2 consumption all fell to a very low value, and the R.Q. became 0.91. Following the convulsions the oxyhemoglobin returned to nearly its normal value, ventilation, expired CO_2, and O_2 consumption were all increased far beyond the normal, while the R.Q. fell slightly to 0.85.

INSULIN IN TISSUES OTHER THAN PANCREAS.

By C. H. BEST, R. G. SMITH, and D. A. SCOTT.

(From the Insulin Division, Connaught Laboratories, University of Toronto, Toronto, Canada.)

Very soon after the isolation of insulin from the degenerated pancreas of the dog, the discoverers attempted to prepare it from other tissues of this animal. The results obtained with liver and spleen were negative, while those with thyroid or thymus were inconclusive. The demonstration of insulin in blood has been reported by Best, Scott, and Banting, and two of us have reported its presence in various other tissues.[18] We have obtained results which furnish additional proof that the active material present in tissues other than the pancreas is insulin. The active material produces a marked lowering in the blood sugar of normal rabbits and diabetic dogs. It enables glycogen to be deposited (approximately 6 per cent) in the livers of completely depancreatized dogs. The general condition of these dogs is greatly improved by the therapeutic administration of insulin containing extracts of blood or thymus gland. Extracts of tissue other than pancreas have on numerous occasions produced typical insulin convulsions in normal rabbits. The convulsions are alleviated by dextrose.

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[18] Best, C. H., and Scott, D. A., *J. Am. Med. Assn.*, 1923, lxxxi, 382.

Insulin is present in reduced amounts in the tissues of completely diabetic dogs. The extracts of diabetic tissues produced the typical insulin effects on normal rabbits and on diabetic dogs. The amount of insulin in diabetic tissues by the method we have used is more than half that obtained by the same method from normal tissues. Obviously, the insulin must exist in diabetic tissues in a comparatively unavailable form.

We have analyzed various tissues from six normal and six diabetic dogs. Our results indicate that all the muscle tissue of the animal contains at least twenty times as much insulin as the total weight of pancreatic tissue. The blood of a dog appears to contain at least five times as much insulin as the pancreas. In some experiments the liver tissue of an animal yielded nearly as much insulin as was extracted from the pancreas by the same method. The active blood sugar reducing principle was extracted from brain, submaxillary, thyroid, and thymus glands, heart muscle, blood, lung, liver, spleen, kidney, bone, and skeletal muscle and, as previously reported, from the urine.

We have been able to keep a diabetic dog, in which less than 1/800 (0.018 gm.) part of its pancreas was found at autopsy, alive for 8 weeks. No insulin was administered and no dietary precautions observed. Our calculations show that the blood of the animal contained approximately 15 units of insulin at the end of this 8 week period.

The tissues of animals which have been subjected to ether anesthesia appear to contain very much less insulin than normal or diabetic tissues. This is interesting in view of the well known effect of ether upon carbohydrate metabolism.

ON THE PURIFICATION OF INSULIN.

By H. A. PIPER, R. S. ALLEN, and JOHN R. MURLIN.

(From the Physiological Laboratory of the University of Rochester, Rochester.)

From extracts of pancreas prepared by perfusion and percolation we have been able invariably to secure a potent preparation of insulin associated with a grayish white precipitate which gives none of the ordinary reactions for protein (biuret, Millon's, xanthoproteic, Hopkins-Cole, etc.). From extracts, also, prepared according to a technique which will be described, we have obtained the active material in this form but not invariably. The determining factors seem to be: (1) the amount of extraneous proteins extracted; (2) the exact pH at which these proteins are precipitated; (3) the reaction at which the insulin together with a residue of proteins is precipitated with sodium chloride; and (4) the reaction at which concentration and final precipitation are effected. The precipitate, when it contains the active agent is quite insoluble in distilled water, and in acid up to 0.5 N HCl, but is readily soluble in very weak alkali. It readily gives up insulin to the body fluids when injected subcutaneously.

The yield of crude insulin is enormously increased by reextraction of the precipitates. In spite of all precautions, however, much of the insulin is lost in process of purification.

THE RESPIRATORY QUOTIENTS OF NORMAL RABBITS AFTER THE ADMINISTRATION OF INSULIN.

By ESTELLE E. HAWLEY and JOHN R. MURLIN.

(From the Physiological Laboratory of the University of Rochester, Rochester.)

The findings of Dudley, Laidlaw, Trevan, and Boock[19] regarding the effects of insulin upon the output of carbon dioxide and the absorption of oxygen are in accord with effects upon the total respiratory exchange occasionally observed in this laboratory[20] when preparations of crude insulin were given to depancreatized dogs. Dudley's experiments did not permit of a determination of the respiratory quotient, hence they do not warrant the conclusion that more carbohydrate (relatively) is not consumed after insulin. In the experiments referred to with the diabetic dog the R.Q. was invariably higher when the blood sugar was markedly lowered and it was often possible from analysis of the urine to state just how much glucose had been utilized (by combustion or conversion). The interpretation provisionally held, but not published, with regard to the lower total metabolism observed at times was that depressor substances were responsible since only partially purified insulin was used with the animals. Many fatal results upon rabbits have been seen when protein precipitates containing no glucopyretic substance were injected. The object of the present series of experiments was to see whether a purer insulin would produce higher quotients without a depression of the total metabolism and whether a definite impurity which raises blood sugar (*glucagon*) would depress the respiratory quotient. The evidence to date is that both these suppositions are correct. The threshold for effect on the respiratory quotient, however, is not the same as the threshold for effect on blood sugar.

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[19] Dudley, H. W., Laidlaw, P. P., Trevan, J. W., and Boock, E. M., *J. Physiol.*, 1923, lvii, p. xlvii.

[20] Murlin, J. R., Clough, H. D., Gibbs, C. B. F., and Stone, N. C., *Am. J. Physiol.*, 1923, lxiv, 348; Murlin, J. R., Clough, H. D., Gibbs, C. B. F., and Stokes, A. M., *J. Biol. Chem.*, 1923, lvi, 253.

ON THE PREPARATION AND PROPERTIES OF INSULIN.

By M. SOMOGYI, E. A. DOISY, and P. A. SHAFFER.

(From the Laboratory of Biological Chemistry, Washington University School of Medicine, St. Louis).

Improvements in the method of preparation described a year ago[21] materially simplify and shorten the procedure, increase the yield and purity of the product, and decrease its cost in time and reagents. The three essential points in our earlier method, and forming the basis of the improved procedure, are: (1) The use of large amounts of strong acid during extraction, which insures solution of the insulin and prevents destruction of proteolytic enzymes; (2) precipitation of the active material by ammonium sulfate; and (3) the precipitation of insulin from semicrude solution by adjusting the reaction to about pH 5.

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[21] Doisy, E. A., Somogyi, M., and Shaffer, P. A., *J. Biol. Chem.*, 1923, lv, p. xxxi.

Fresh beef pancreas is finely hashed by passing twice through a motor driven meat grinder. To each kilo of hash are added 20 cc. of 10 N sulfuric acid, and after standing at room temperature from 4 to 12 hours with occasional stirring the extract is filtered through paper (without neutralization). The filtration is rapid. The residue is pressed in a power press, and the press-liquid is filtered. The combined extract is evaporated at low temperature.

When the odor of alcohol is gone, and the volume reduced to about one-tenth (or possibly less) the liquid is allowed to cool to about 20°C. and poured on moistened filter paper. The separated fats are thus completely removed with some protein, while at the reaction of the solution (pH 3 to 4) the insulin remains in the solution.

The clear filtrate is precipitated by half saturation ammonium sulfate, and the active material is separated from the solution of this precipitate by adjusting the reaction to about pH 5.

This first "isoelectric precipitate" contains at least three proteins which may be separated by fractionating at different reactions in alcoholic or aqueous solutions. The active fraction is precipitated best near pH 5, and has been purified to the point that 0.02 or 0.03 mg. of dry material represents a standard Toronto unit. The solubility curves of these protein fractions with changing pH were presented.

FURTHER PURIFICATION OF INSULIN AND ANALYSIS OF THE PRODUCT.

By EDWARD A. DOISY and CLARENCE J. WEBER.

(*From the Department of Biological Chemistry, St. Louis University School of Medicine, St. Louis.*)

Fractionation of crude insulin employing alcohol as solvent and the phenomenon of isoelectric precipitation have yielded a product the activity of which lies between 0.015 to 0.025 mg. per Toronto unit (one-third the quantity for a 2 kilo rabbit).

This purified insulin gives a biuret reaction corresponding to an equivalent weight of peptone. By colorimetric procedures, the total nitrogen is 14 per cent. After hydrolysis the ammonia nitrogen is 10 per cent, and the amino nitrogen 70 per cent of the total nitrogen. The tryosine content is 10 per cent and the cystine 13 per cent. Tryptophane is absent. These figures are only approximate.

Characteristic isoelectric behavior has been observed with solutions containing only 0.05 mg. per cc.

The purified product is rapidly inactivated by trypsin.

SOME EFFECTS OF INSULIN ON THE CARBOHYDRATE AND PHOSPHORUS METABOLISM OF NORMAL INDIVIDUALS.

By N. R. BLATHERWICK, MARION BELL, and ELSIE HILL.

(*From the Chemical Laboratory, Potter Metabolic Clinic, Santa Barbara Cottage Hospital, Santa Barbara.*)

1 hour after glucose is taken in tolerance tests (Janney) there is a perceptible drop in the inorganic P of blood plasma. When 40 rabbit units of insulin are given before the glucose there is usually a marked drop in the plasma phosphate and in the excretion of urine P at the time of hypoglycemia. In some cases, the plasma phosphate may first increase and then decrease.

The hourly rates of excretion of P and sugar of two normal individuals living upon a basal diet were determined. During the forenoon the P curve fell and the sugar curve rose, but during the remaining 24 hours they were quite similar, both reaching a peak in the 6 to 8 p.m. period. Insulin in equal doses before breakfast and supper (from 7 to 48 kilo rabbit units in increasing amounts were given) caused an increased excretion of P for 2 and 3 days, respectively. Thereafter the morning rate was markedly decreased. One subject then responded with a decreased rate of excretion for the entire 24 hours. The other subject finally showed a decreased excretion in the rate during the day, but an increased night rate kept the total amount above that for the basal diet. The difference in response of the two subjects is probably referable to the carbohydrate reserves of the body.

The fermentable sugar of the urine decreased by 12 and 28 per cent when insulin was given.

The bearing of these observations upon the formation of the hypothetical hexose diphosphate combination in intermediary carbohydrate metabolism was discussed.

SOME FACTORS INFLUENCING THE RESPONSE OF RABBITS TO INSULIN.

By N. R. BLATHERWICK, M. LOUISA LONG, MARION BELL, L. C. MAXWELL, and ELSIE HILL.

(*From the Chemical Laboratory, Potter Metabolic Clinic, Santa Barbara Cottage Hospital, Santa Barabara.*)

Data were presented which showed that rabbits fed the low carbohydrate, base-forming diet of alfalfa hay are less resistant to insulin than are rabbits fed the high carbohydrate, acid-forming diet of alfalfa hay and crushed barley. The greater resistance of animals fed the latter diet appears not to be due to an increase in the glycogen content of the tissues, since animals maintained on both diets and fasted for 24 hours showed practically the same amounts of glycogen in the liver and the muscles.

An "education" to insulin convulsions was also demonstrated; *i.e.*, convulsions are more easily produced after rabbits have had one or more convulsions.

Experiments were cited which indicate that the same amount of insulin per kilo of body weight is effective in producing convulsions even after the rabbits have doubled in weight. These results support the hypothesis that the dosage of insulin varies directly with the body weight and not as the square of the weight as was the belief of some.

In this laboratory, our rabbits are fed a diet of alfalfa hay and are fasted for 24 hours before the insulin test. If no convulsion results from a given dose more insulin is given to produce this result. After convulsions the animals are fed crushed barley in addition to the alfalfa hay; also the following day. The crushed barley is added to replenish the carbohydrate stores of the body. The alfalfa hay must contain a goodly proportion of leaves to be satisfactory. Growing rabbits must gain steadily in weight in order to give reliable results. Our animals are used every 7th day. Rabbits which have never received insulin are given sufficient amounts to produce convulsions before they are used for standardization purposes. This is done to "educate" the animals.

OBSERVATIONS ON INTERMEDIATE CARBOHYDRATE METABOLISM BY MEANS OF INSULIN.

By CARL VOEGTLIN, EDITH R. DUNN, and J. W. THOMPSON.

(From the Division of Pharmacology, Hygienic Laboratory, United States Public Health Service, Washington.)

A reliable method for the physiological standardization of insulin has been worked out which is based on the lethal effect produced by the drug in standardized albino rats kept under standard conditions of diet and atmospheric temperature. This method was used to determine the antagonistic effect (against insulin) of certain substances which are generally supposed to be formed in the breakdown of carbohydrates or substances which are believed to be convertible into glucose within the mammalian body. The results obtained indicate that, with certain limitations, the method is quite satisfactory for the study of intermediate carbohydrate metabolism.

THE OCCURRENCE OF A HYPOGLYCEMIA-PRODUCING SUBSTANCE IN BACTERIA.

By JOHN T. LITTLE, VICTOR E. LEVINE, and CHARLES H. BEST.

(From the Biochemical Laboratory, School of Medicine, Creighton University, Omaha, and the Connaught Laboratories, Insulin Division, University of Toronto, Toronto, Canada.)

Bacteria are grown in a culture medium free from substances that may contain insulin such as peptone, meat, blood, etc. The simple medium used contains 1 gm. of dipotassium phosphate, 1 gm. of magnesium sulfate, and 2 gm. of asparagine per liter of distilled water. This liquid medium is easily sterilized by boiling. Certain bacteria such as *B. coli* and *B. subtilis* grow very readily in it.

Four flasks each containing 250 cc. of the medium are inoculated and the organisms allowed to grow at 37.5°C. for 4 to 7 days. The medium is then diluted with alcohol so that the content of the latter is 75 per cent, enough sulfuric acid is added to make a concentration of 0.5 per cent. The acid mixture is now allowed to remain at room temperature or at 37.5°C. until upon neutralization, straining, and microscopic examination, the organism has been found to undergo morphological disintegration. The precipitate which collects at the bottom of the flask is now filtered off and discarded. The hypoglycemia-producing substance is precipitated with 1.25 volumes of ether. After standing 2 or 3 days a precipitate, sometimes of solid consistency and sometimes gelatinous, forms at the bottom. This is removed from the ether by means of a separating funnel and washed three times with 100 cc. of a mixture of equal volumes of 95 per cent alcohol and ether. The washed precipitate, which is very small in quantity is allowed to dry in the air for a few minutes. The precipitate is finally dissolved in 10 cc. of distilled water having a pH of 2.5 and filtered before injection.

The organisms so far tested yield a substance giving a negative biuret and having the power to lower the blood sugar, and to keep the sugar at a low level for a long period. An illustration of the effect on the rabbit of the bacterial hypoglycemic substance (from *B. subtilis*) is appended:

Blood sugars.	
	per cent
Normal	0.136
1 hr. after injection	0.072
2 hrs. " "	0.054
3 hrs. " "	0.048
4 hrs. " "	0.060

HISTAMINE AS A CONSTITUENT OF SECRETIN PREPARATIONS.

By ELOISE PARSONS and FRED C. KOCH.

(From the Hull Laboratories of Physiological Chemistry, University of Chicago, Chicago.)

Comparative chemical and physiological studies on dogs with secretin preparations and pure synthetic histamine led the authors to the following conclusions:

1. Secretin and histamine are similar both chemically and physiologically.

2. The secretin, unlike histamine, is not quantitatively recovered by the phosphotungstic acid precipitation.

3. Secretin, unlike histamine, is not extracted by amyl alcohol in alkaline solution.
4. The depressor and secretagogue actions of secretin and histamine, respectively, are not of the same quantitative order. The former is the better secretagogue and the latter the better depressor.
5. The secretin preparations when properly prepared do not contain histamine in amounts which are of physiological significance.
6. By chemical and physiological assays it was shown that neither secretin nor histamine is absorbed from the duodenal loop and neither is appreciably destroyed by such treatment.
7. The results suggest that secretin preparations contain two activities, a secretagogue and a depressor substance.

THE CHEMICAL REACTIONS INVOLVED IN THE PHYSIOLOGICAL FUNCTIONING OF THYROXIN.

By E. C. KENDALL.

(From the Section of Biochemistry, Mayo Clinic and Mayo Foundation, University of Minnesota, Rochester.)

During the synthetic work leading to the elaboration of thyroxin, a derivative was produced which differed from thyroxin in having one more bond and two less hydrogens. Both of these forms occur in the thyroid gland. The functioning of thyroxin in the tissues is brought about by the molecule oscillating between these two forms. The function of thyroxin is to regulate the capacity of a cell to use oxygen.

The rate of functioning of thyroxin in the tissues is dependent on the physical state of the cell. The alternate oxidation and reduction is regulated by the opening and closing of the pyrrole ring in the tissues. This opening and closing of the ring is dependent on the concentration of positive and negative charges present. It therefore appears probable that thyroxin makes any cell more sensitive to the external, that is, physical conditions.

A QUANTITATIVE ESTIMATE OF THE CATALYTIC POWER OF ADRENALIN AND THYROXIN AS CALORIGENIC AGENTS AND THE RELATIVE RATE OF THEIR DESTRUCTION.

BY WALTER M. BOOTHBY AND IRENE SANDIFORD.

(*From the Section of Clinical Metabolism, Mayo Clinic and Mayo Foundation, University of Minnesota, Rochester.*)

The administration of both adrenalin and thyroxin produces in the human an excess of heat over and above that which would have been produced had not the substance been given. It is possible to construct plots integrating this increased heat production for given weights of thyroxin and adrenalin. For example, we find in one experiment that 16 mg. of thyroxin injected intravenously in a myxedematous (thyroidless) individual produces above the basal 16,125 calories, or 1,008 calories, per mg. or 589,680 calories per mg. mol. For 0.5 mg. of adrenalin injected subcutaneously there is produced above the basal 25 calories, or 50 calories per mg. or 9,150 calories per mg. mol. Therefore, the relative effectiveness of a molecule of thyroxin as a catalytic agent in the production of heat is 64 times that of an adrenalin molecule.

The various points of the descending limbs of the calorigenic curves of both adrenalin and thyroxin when plotted on semilogarithmic paper show a remarkable tendency to lie upon a straight line. In a large proportion of the experiments the points are within ± 5 per cent of such a line and in a few are directly upon it. This is consistent with an interpretation that the system suffers an essential change of concentration of only one molecular species and that, therefore, the rate of excess heat production is dependent directly upon the concentration of thyroxin or adrenalin in the tissues. The duration of time for the decrease in heat production from the maximum to one-tenth of this amount is found by extrapolation to be usually, in the case of adrenalin, between 1 and 4 hours and for thyroxin between 30 and 70 days.

THE EFFECT OF THYROID AND PARATHYROID DEFICIENCY ON THE GROSS CHEMICAL COMPOSITION OF THE LONG BONES.

By FREDERICK S. HAMMETT.

(From The Wistar Institute of Anatomy and Biology, Philadelphia.)

When the thyroid apparatus is removed from albino rats 100 days of age a distortion of the course of differential development in gross chemical composition is exhibited. The nature of the disturbance consists of a combined partial desiccation and a retardation of ossification. The desiccation is shown by the fact of an actual loss of water from the bones. The retardation of ossification is shown by the decrease in percentage of ash. The changes are more marked in the humerus than the femur and in the bones of the females.

When the parathyroid glands are removed there is no alteration in the course of differential development in gross chemical composition in the males. In the females an evident retardation of ossification is produced, but no drift towards desiccation.

THE METABOLISM IN PREGNANCY.

II. CHANGES IN THE BASAL METABOLIC RATE.

By A. W. ROWE, M. D. ALCOTT, and E. MORTIMER.

Scattered through the literature, nine cases are recorded, more or less completely studied for the basal metabolic rate during pregnancy; that of Root, with one case, being the latest and most complete. In addition, there are two clinical papers by Baer and Cornell, but the studies are too fragmentary and too much influenced by the psychic factor to require discussion. The present report deals with the careful study of two series of cases.

Series I.—This series was comprised of patients from a large hospital prenatal service, and from private practice.

Series II.—These were cases from an institution for unmarried mothers. The basal metabolism was determined by the use of Benedict respiration units of the closed circuit type. Every precaution offered by control measurements was observed to secure a maximum of accuracy, in both the main measurements and the subsidiary observations. The objective findings may be tabulated as follows, the recorded figures being averages. The relative basal metabolic rate is determined by comparison of the observed rate with the average of the Harris-Benedict and Du. Bois standards.

TABLE I.

	Series I.	Series II.	
No. of cases	25	21	
Age, *yrs*	28	18	
Weight of child, *kilos*	3.31	3.20	
Partum.	Ante.	Ante.	Post.
Weeks studied	21	8	5
Blood pressure (systolic)	106	115	114
" " (diastolic)	65	76	75
Body temperature, °*C*	98.0	97.6	97.9
Pulse rate	78	82	67
Respiration rate	17	19	19

TABLE II.

Weekly Rate of Change (Antepartum).

	Series I.	Series II.
Weight, *kilos*	+0.32	+0.52
Vital capacity, *per cent*	+0.59	
Basal metabolism (absolute) *per cent*	+0.90	+1.02
" " (relative) " "	+0.53	+0.60
Difference (absolute−relative)	+0.37	+0.42

SUMMARY.

1. There is an increase in the basal energy output. Further, this is in excess of that conditioned by weight increase.

2. The vital capacity increases.

3. The changes seem to be linear in character and are manifest as early as 30 weeks before delivery.

THE DECOMPOSITION OF GLUCOSE BY BACTERIA.

By E. GORDON YOUNG.

(*From the Biochemical Laboratory, University of Western Ontario, London, Canada.*)

Fermentations of pure glucose by a stock laboratory strain of *B. coli communis* yielded the variety and proportion of fermentation products obtained from other strains after gross sowings. By the Chambers micro manipulator single cell isolations have been made and fermentations carried out on these pure line strains. Several types of fermentation resulted, differing in the quantitative aspect of the products formed; lactic acid and carbon dioxide, or acetic and succinic acids predominating. This is taken to suggest that the ordinary bacteriological strain of *B. coli communis* contains cells possessing differing types of metabolism. It is further suggested that glucose may be broken down in fermentation by several different paths of decomposition.

PREPARATION AND PROPERTIES OF AN OVARIAN HORMONE.

By EDWARD A. DOISY, EDGAR ALLEN, J. O. RALLS, and C. S. JOHNSTON.

(*From the Department of Chemistry, St. Louis University School of Medicine, St. Louis.*)

A hormone which causes typical estrus in spayed rats has been prepared from liquor folliculi and whole ovaries. Extraction of the lipoids with hot alcohol (95 per cent) is followed by hydrolysis with sodium hydroxide and evaporation to dryness. The hormone is subsequently extracted from the soaps with chloroform or ether. The chief contaminating substance at this stage is cholesterol. Separation by fractional crystallization has been unsuccessful, but the precipitation of the cholesterol by digitonin does not remove the hormone.

The hormone is not cholesterol and does not give cholesterol color reactions. It is soluble in lipoid solvents but insoluble in water. It is stable toward dilute boiling acids and alkali. Dissolved in oil, its activity is not destroyed by autoclaving at 15 pounds pressure for 15 minutes.

Preparations from placenta, liquor folliculi, and ovaries from which the liquor folliculi have been removed, are active; from embryos and corpora lutea, negative.

NUTRITION AND GROWTH ON DIETS HIGHLY DEFICIENT OR ENTIRELY LACKING IN PREFORMED CARBOHYDRATES.

BY THOMAS B. OSBORNE AND LAFAYETTE B. MENDEL.

(From the Laboratory of the Connecticut Agricultural Experiment Station and the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.)

White rats were given diets in which the amount of available *preformed* carbohydrate was so small as to be undetectable. The foods contained various proteins and fats in widely different proportions, along with inorganic salts and sources of vitamines A and B. When the energy was furnished essentially in the form of protein rats grew well to 250 gm. or more. The food intake was not notably large, indicating that "protein calories" are well used. Diuresis and hypertrophy of the kidneys were often noted. Microscopic examination failed to disclose inflammation or degenerative changes. On diets containing different proteins and varying proportions of fats with the other dietary essentials but without preformed carbohydrate, good growth was secured in many instances to large size. The absence of preformed carbohydrate did not prevent or alter the prompt recovery of rats which had declined on foods containing no vitamine A, when this factor was supplied. Rats also grew on diets in which the organic foods consisted essentially of protein and fatty acids. The presence of glycerol did not produce any noteworthy advantage. In some of the experiments with foods extremely rich in fats, without apparent detriment to the animals, the ketogenic-antiketogenic ratio greatly exceeded the limit beyond which ketosis is said to arise in man.

Our experiments indicate that in as far as carbohydrate is required for the intermediary metabolism, particularly for the metabolism of fats and the development of energy in muscular contraction, it can be furnished endogenously throughout the period of growth to adult size.

A complete account will appear in the February issue of the Journal of Biological Chemistry.

GROWTH AND REPRODUCTION UPON SIMPLIFIED FOOD SUPPLY. IV.

By H. C. SHERMAN and H. L. CAMPBELL.

(From the Department of Chemistry, Columbia University, New York.)

Rats were fed mixtures of ground whole wheat and dried whole milk with sodium chloride and distilled water. Starting with a diet which was already adequate for both growth and reproduction, it was found that a change in the quantitative proportions of the food mixture resulted in improved nutrition as evidenced by more rapid and economical growth, earlier maturity, greater success in reproduction and lactation, and postponement of senility. Criteria for the quantitative measurement and interpretation of such differences in nutrition are considered.

FAT-SOLUBLE VITAMIN IN ADULT NUTRITION.

By H. C. SHERMAN and F. L. MacLEOD.

(From the Department of Chemistry, Columbia University, New York.)

Two groups of rats of identical family histories have been kept under conditions, alike in other respects, but with different amounts of fat-soluble vitamin in their food. The smaller allowance of fat-soluble vitamin has sufficed to support normal growth to adult size with apparent good health throughout the youth of the animals, but has failed to support normal reproduction and suckling of the young, whereas in the parallel group receiving a more liberal allowance of fat-soluble vitamin, reproduction and success in lactation were fully normal. The more liberal allowance of fat-soluble vitamin has also resulted in a greatly increased length of life.

STUDIES IN PARENCHYMATOUS NEPHRITIS.

By S. W. CLAUSEN.

(From the Washington University, School of Medicine, St. Louis.)

Parenchymatous nephritis ("nephrosis") is a widespread systemic disorder characterized by albuminuria and edema, without increase of blood pressure or retention of nitrogenous waste. In a series of twenty such cases, a chronic nasal sinus staphylococcus infection could be shown to be the cause in most instances.

The plasma proteins were invariably low, and the colloidal osmotic pressure correspondingly low. This factor may contribute to the edema.

The surface tension of the plasma (or serum) as determined by the drop-weight method is invariably low in contrast to the remarkable constancy in normal individuals. The degree of lowering accurately reflects the clinical progress of the cases. In no other disease excepting nutritional edema is such marked lowering found.

The surface active material may be prepared from the urine as follows: (1) evaporation at low temperature; (2) dialysis; (3) desiccation of colloidal residue; and (4) extraction of dry colloids with 95 per cent alcohol. The dry extract is a wax, swelling in water, soluble in alkali, and precipitated most completely at a reaction of pH 3.5. It is insoluble in ether or benzene. It is salted out from urine and plasma chiefly in the albumin fraction. Animal charcoal removes it completely from these fluids.

Collodion membranes prepared from 6 per cent pyroxylin are normally impermeable to proteins. When first treated with the waxy material in water, or with urine or plasma from cases of parenchymatous nephritis, the membranes become readily permeable.

A VOLUMETRIC METHOD FOR THE QUANTITATIVE DETERMINATION OF SODIUM IN SMALL AMOUNTS OF SERUM.

By BENJAMIN KRAMER and I. F. GITTLEMAN.

(From the Department of Pediatrics, the Johns Hopkins University, Baltimore.)

2 cc. of serum are evaporated to dryness on a steam bath and ashed according to the method of Stolte.[22] After cooling, the ash is dissolved in exactly 2 cc. of 0.1 N hydrochloric acid and the solution is made alkaline to litmus by adding 10 per cent potassium hydroxide (alcohol-washed), drop by drop. Exactly 10 cc. of potassium pyroantimonate reagent[23] are then added, followed by 3 cc. of 95 per cent alcohol. If precipitation does not begin at once it may be accelerated by stirring with a rubber tipped glass rod. The platinum dish is covered to prevent evaporation. At

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[22] Stolte, K., *Biochem. Z.*, 1911, xxxv, 104.

[23] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, xlvi, 467.

the end of a half hour the contents of the dish are poured into a paraffined 50 cc. centrifuge tube. This is centrifuged for a few minutes and the supernatant fluid is then siphoned off. A 10 cc. aliquot is measured into a beaker. Exactly 5 cc. of concentrated hydrochloric acid are added and the beaker is rotated until the precipitate, which first forms, goes completely into solution. 20 cc. of a 20 per cent solution of potassium iodide are then added. Free iodine is formed and colors the solution a deep brown. The sample is then rapidly titrated with 0.1 N sodium thiosulfate until the brown color begins to disappear. 1 cc. of 1 per cent solution of soluble starch is then added and the titration continued with thorough stirring of the sample between additions until the brown color completely disappears, leaving a clear yellow solution. A similar titration is carried out on 10 cc. of the reagent (potassium pyroantimonate).

The calculation is made as follows:

1 cc. of 0.1 N sodium thiosulfate = 6 mg. of antimony.

1 mg. of antimony = 0.192 mg. of sodium.

(1). The number of cc. of thiosulfate used for 10 cc. of reagent × 6 = mg. of antimony in reagent.

(2). The number of cc. of thiosulfate required for a 10 cc. aliquot of supernatant fluid × 6 × $\frac{3}{2}$ = mg. of antimony remaining in the supernatant fluid uncombined with sodium.

The difference between (1) and (2) = mg. of antimony in precipitate.

This multiplied by 0.192 = mg. of sodium in the sample × 50 = mg. of sodium per 100 cc. of serum.

THE CHEMISTRY OF JAFFE'S REACTION FOR CREATININE.

By ISIDOR GREENWALD.

(From the Harriman Research Laboratory, the Roosevelt Hospital, New York.)

After acidifying the mixture of creatinine, picric acid, and sodium hydroxide, both creatinine and picric acid can be quantitatively recovered. Only 1 mol of picric acid appears to be required for each mol of creatinine, although the reaction is not complete unless an excess of picric acid is present. The change from red to yellow, upon acidifying, is not immediate, indicating that the alkali is not an essential part of the colored molecule. The red color seems to depend upon an internal rearrangement within the molecule of creatinine picrate, possibly with the absorption of water. The reaction is not given by a number of substances chemically more or less similar to picric acid.

THE EFFECT OF MANGANESE ON GROWTH.

By VICTOR E. LEVINE AND HERBERT A. SOHM.

(*From the Biochemical Laboratory, School of Medicine, Creighton University, Omaha.*)

Plant and animal cells are known to contain small quantities of such elements as copper and manganese, and some lower organisms contain vanadium. These elements, like iron, possess more than one valency and are characterized by marked catalytic properties. Their occurrence in living substance argues for some important function on their part. With this view in mind, experiments with minute quantities of these elements have been undertaken in relation to growth. The experiments with manganese illustrate the method pursued.

The experiments were run in units. In order to secure young rats having the same prenatal nutritional and growth tendencies each unit was made up of all males or females from the same litter. One or two rats served as controls, while the others were given varying concentrations of manganese sulfate, which in terms of manganese represented dilutions of 1:2,000, 1:4,000, or 1:10,000.

The diet used was the McCollum polyneuritic diet (vitamin B-free casein 18 per cent, dextrin 71.3 per cent, agar-agar 2 per cent, salt mixture 3.7 per cent, and butter fat 5 per cent). The sodium chloride of the salt mixture carried an iodine content of 0.03 per cent. Orange juice was employed as a source of vitamin B and this was served in the ratio of 10 cc. to 40 cc. of distilled water or water containing manganese sulfate. The initial weight of the rats used varies from 20 to 50 gm. The control animals, but particularly the manganese rats, for the first 3 or 4 weeks suffered from severe diarrhea. During this period the animals lost weight or made very slight gain in weight or none. Occasionally the diarrhea proved severe enough to cause death.

The results of the experiments indicate that the manganese has a marked catalytic effect on growth. The manganese rats proved more active than the controls, their coats of hair were sleeker, longer, and thicker. These facts are in harmony with the power of manganese salts to accelerate oxidations such as take place with unsaturated oils or in the biologic organism, to increase such biologic activities as autolysis, the formation of antitoxin,[24] the growth of plants,[25] or the healing of wounds.[26]

In view of the findings of Evans and Bishop[27] in reference to a dietary factor essential for reproduction it was surprising to find that in a few preliminary experiments the manganese rats proved fertile and that the offspring of the manganese rats grew somewhat faster than the average young rat. The only source of the reproductive vitamin in the diet may possibly be the milk products, casein, or butter fat, although according to Evans and Bishop the levels of intake employed for either casein or butter fat would not warrant the assumption of the presence of a factor necessary for normal reproductive function.

THE UTILIZATION OF MARGARIC ACID ESTERS.

By ELMER L. SEVRINGHAUS.

(*From the Laboratory of Physiological Chemistry, University of Wisconsin, Madison.*)

Two healthy adults were fed 2,500 calorie diets, made up of normal foods low in fat and 100 gm. of glyceryl margarate. Beef suet was used similarly as a control. The fat in the stools was determined by titration of free fatty acid and saponification of neutral fat. The beef fat was utilized to the extent of 97.4 to 97.8 per cent. The glyceryl margarate showed 96.5 to 96.8 per cent absorption. There was a slight increase in the neutral fat of the stools when margarate was fed. "Intarvin" was utilized as well as glyceryl margarate.

The same subjects were fed a diet low in protein and almost free from carbohydrate and fat. After ketonuria had become pronounced glyceryl margarate was added in increasing amounts up to 100 gm. daily. There was no diminution in the ketonuria.

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[24] Walbum, L. D., and Mörch, J. R., *Ann. Inst. Pasteur*, 1923, xxxvii, 396.

[25] McHargue, J. S., *J. Agric. Research*, 1923, xxiv, 781.

[26] McDonagh, J. E. R., *Proc. Roy. Soc. Med.*, Dermatol. Sect., 1923, xvi, 66, 70.

[27] Evans, H. M., and Bishop, K. S., *J. Am. Med. Assn.*, 1923, lxxxi, 889.

The addition of 50 and 75 gm. of carbohydrate promptly reduced ketonuria. Glyceryl margarate seems to have little or no ketolytic value aside from its glycerol content. It does exert a protein-sparing action in these diets.

A NEW DISTINCTIVE TEST FOR CYSTEINE.

By M. X. SULLIVAN.

(From the Division of Chemistry, Hygienic Laboratory, United States Public Health Service, Washington.)

On the addition of one-tenth volume of normal NaOH to a mixture of 1.2 naphthoquinone-4-sodium sulfonate and amino acids and various thio compounds there is speedily developed a color varying from reddish orange to dark brown, dependent on the concentration of the amino acids, etc. Of the colors thus formed, that given by cysteine is the only one not discharged by sodium hydrosulfite, $Na_2S_2O_4$. Cysteine, unreduced, behaves like other amino acids. On the addition of the hydrosulfite, the brownish red color given by cysteine becomes a finer red. If 5 cc. of a solution of various amino acids, containing approximately 0.07 mg. of N per cc., are treated with 0.3 cc. of a 0.5 per cent solution of the naphthoquinone monosulfonate and then with 5 cc. of a 20 per cent solution of sodium sulfite in 0.25 N NaOH, the cysteine (used as cysteine hydrochloride) alone gives the brilliant red color. The colored compound formed by the union of cysteine and the naphthoquinone monosulfonate is apparently a hydroquinone derivative. Work on the quantitative application of the reaction is in progress.

SURFACE TENSION AND THE DETOXICATION OF FOREIGN ORGANIC SUBSTANCES.

By A. R. ROSE and C. P. SHERWIN.

(From the Department of Chemistry, Fordham University, New York.)

In the study of detoxication a large number of substances have been synthesized or recovered from the urine and purified. The surface tensions of these are being determined with the twofold purpose: first, to see if the surface tensions and the mode of detoxication can be correlated; and second, to find a rapid means of selecting the probable structure of two or more possibilities in preparations recovered from urines. The observations of Berczeller on benzoic acid have been confirmed. With some exceptions the detoxicated products have a lesser effect on depressing the surface tension of water than their precursors. This relation is particularly noticeable in the direct readings of saturated solutions, but less so and sometimes obliterated or even reversed when calculated to a millimolar basis.

THE ABSORPTION OF SODIUM BENZOATE AND OF SODIUM HIPPURATE FROM THE SMALL INTESTINE OF THE RABBIT.

By WENDELL H. GRIFFITH.

(From the Laboratories of Physiological Chemistry of the University of Michigan, Ann Arbor, and of the St. Louis University School of Medicine, St. Louis.)

In order to use the quantity of hippuric acid excreted in the 6 hour period after the administration of benzoate as an index of the rate of synthesis of hippuric acid, the rate of absorption of the benzoate from the intestine must be ruled out as a process which might influence the output of hippuric acid in the experimental period. Direct determinations were made of the rate of absorption of sodium benzoate and of sodium hippurate from intestinal loops. In these experiments benzoate was found to be absorbed from two to four times as rapidly as the hippurate. After the oral administration of sodium hippurate in quantities equivalent to 1 gm. of benzoic acid per kilo, 51 per cent was excreted in the 6 hour period. Since this represented the rate of absorption of hippurate from the intestine, and since in the loop experiments benzoate was found to be absorbed from two to four times as rapidly as equivalent amounts of hippurate, it was concluded that the rate of absorption of benzoate was sufficiently rapid to permit a high rate of synthesis of hippuric acid after the oral administration of benzoate with a suitable precursor of glycine.

The rate of excretion of sodium hippurate from the kidneys after its oral administration was found to be markedly increased by the simultaneous oral or subcutaneous administration of glycine. Similar results were obtained after the ingestion of hippurate with gelatin or elastin, proteins which have a high content of glycine.

Little or no effect was observed after the administration of hippurate with alanine or proteins which have a low content of glycine, such as egg albumin, casein, edestin, and glutenin. These experiments with others, made it seem probable that the hippuric acid was largely hydrolyzed to benzoic acid in the intestine. The higher rate of excretion after the administration of hippurate with glycine or with proteins containing glycine was considered to be due to a higher rate of conjugation of the benzoic acid with glycine.

PHOSPHOLIPINS IN YEAST.

By W. C. AUSTIN.

(From the Hull Laboratories of Physiological Chemistry, University of Chicago, and the Department of Physiological Chemistry, Loyola University Medical School, Chicago.)

Starch-free baker's yeast contains 4.5 per cent of total lipin and 0.5 per cent of crude phospholipin. A phospholipin with a N:P ratio of unity was made and found to be a mixture of lecithin and cephalin, with the former in predominance. No sphingomyelin was found. The mixture of lecithin and cephalin was fractionated by forming the cadmium chloride salts and recrystallizing the same. Pure lecithin cadmium chloride was prepared. The cephalin decomposed in the process of purification.

Over twenty-five fractions of phospholipin were obtained and analyzed for nitrogen and phosphorus content, and for the nature of the nitrogen-containing substances produced by hydrolysis. The N:P ratios, while near 1:1, were variable, with nitrogen usually greater than 1 in the ratio. The samples yielded choline, cholamine, and varying quantities of unknown nitrogenous substances on hydrolysis. A more accurate method for the estimation of choline was developed.

THE OXIDATION AND REDUCTION OF BILE PIGMENTS.

By WILLIAM M. BARRY and VICTOR E. LEVINE.

(From the Biochemical Laboratory, School of Medicine, Creighton University, Omaha.)

Bilirubin absorbs oxygen from the air and is converted into biliverdin. This oxidation requires a definite hydrogen ion concentration. Yellow-brown ox bile, dog bile (obtained by fistula), or human bile (obtained by surgical procedure) becomes green after standing for some time at a pH of 6.4. At a pH above this (6.5 to 8.0) the bilirubin of the bile does not undergo oxidation to biliverdin. The bile as it leaves the liver has a pH about the same as that of blood or a little higher (7.2 to 7.8). The tendency of the gall bladder is to increase the acidity of the bile, for bile in the bladder has a pH ranging from about 5.0 to 7.2. Various local pathological conditions, such as infection, trauma, low blood supply, and excessive dehydration may bring about a local acidosis and thereby cause the appearance in the gall bladder of a dark green bile.

Green bile on exposure to air is changed to yellow bile. The change is due to bacteria. Green bile or solutions of biliverdin do not change in color when rendered sterile by heating. The reduction of biliverdin itself or green bile is also accomplished at a pH of 7.4 to 7.6 by liver or yeast cells. This action is due to the enzyme reductase. The reducing action is accelerated by glucose. Heating yeast or liver cells destroys their reducing power.

Liver bile in view of its hydrogen ion concentration and the reducing tendencies of liver cells is a bilirubin bile. We have never found fistula bile to be dark (stasis) or green (oxidation). In infectious conditions of liver or gall bladder the local acidosis may overcome the powerful reducing effect of the liver cells or of the invading organisms with the resulting formation even in the liver itself of green bile or calculi containing biliverdin. In view of the changes liable to take place in bile on standing, observation on color in medical drainage should be made soon after the bile has been collected.

THE EFFECT OF THE INGESTION OF GRANULAR COMMERCIAL GLUCOSE ON THE REDUCING SUBSTANCE IN BLOOD AND URINE.

By CHI CHE WANG and AUGUSTA R. FELSHER.

(From the Nelson Morris Memorial Institute for Medical Research, Michael Reese Hospital, Chicago.)

The work was undertaken to determine whether the analysis of hourly specimens of urine might be substituted for tests on blood sugar for diabetic suspects. Thirteen normal adults served as subjects in two sets of experiments, using 1 and 2 gm. of granular commercial glucose per kilo of body weight. One diabetic was given the same tests with 0.4 gm. per kilo.

The curves for reducing substance in blood were similar in the two cases, showing a maximum rise of about 50 per cent above the fasting value, with the peak at the 30 minute period, and a subsequent decline. The amount of increase in reducing substance in the urine is insignificant when 1 gm. per kilo is ingested. With 2 gm., however, the average figures are seven times as high. The similarity of the blood values and the divergence of the urine values seem to indicate that 1 gm. per kilo is near the average threshold value, and that any increase of sugar ingested is eliminated through the urine. The diabetic patient, after ingesting 0.4 gm. of glucose per kilo of body weight, showed a percentage increase in blood sugar not greatly different from the normal, although the peak was somewhat delayed, but the urinary excretion was tremendously greater—more than twice as much as the highest normal subject on one-fifth the amount ingested. This significant difference in excretion between diabetic and normal suggests the possibility of substituting the tests on urine for those on blood under well controlled conditions.

SOME OBSERVATIONS ON SUGAR TOLERANCE, USING GRANULAR COMMERCIAL GLUCOSE, CHEMICALLY PURE GLUCOSE, AND CHEMICALLY PURE GLUCOSE PLUS EXTRACT OF COMMERCIAL GLUCOSE.

By CHI CHE WANG and AUGUSTA R. FELSHER.

(From the Nelson Morris Memorial Institute for Medical Research, Michael Reese Hospital, Chicago.)

Tests were made on the reducing substance in blood and urine of six normal adults after the ingestion of 2 gm. per kilo of body weight of (1) c.p. glucose, (2) granular commercial glucose, (3) c.p. glucose plus alcoholic extract of commercial glucose, (4) extract alone, and (5) control. There was no significant difference in the blood under the first three conditions. The peak occurred in all cases after 30 minutes, and was somewhat higher when extract plus c.p. sugar was used. Extract alone had little effect on the blood sugar.

The values for urine are strikingly different from those for blood. There was little difference between the effect of extract and the control, neither showing significant increase in urinary reducing substance. Both commercial and C.P. glucose showed a peak at the end of 1 hour, but the average increase in the former is nearly three times as great as in the latter. When extract was added to C.P. sugar an even greater quantity was excreted. The average peak was delayed until the end of 2 hours, and was nearly four times as great as that of C.P. glucose alone. Although the amount of sugar contained in the extract is only 1.6 per cent of the total amount ingested, the increased excretion of reducing substance is entirely disproportionate. This suggests that there is some factor in the extract which accelerates the excretion of sugar. It will be interesting to determine the specific factor which exerts this influence. Chemical analyses are now under way, but there are as yet no suggestions to offer.

FURTHER WORK ON DYE-PROTEIN AGGREGATES.

By L. F. SHACKELL.

(From the Physiological Laboratory, University of Utah, Salt Lake City.)

Aggregates of the proteins of egg white with "acid" as well as "basic" dyes have been prepared, which do not lose any dye in passing through a pH range of 2 to 8. The extremes of this pH range are well on the acid and basic sides, respectively, of the isoelectric points of the proteins used. Loeb's contention that acid dyes unite with a protein only on the acid side, and basic dyes unite with the same protein only on the basic side of its isoelectric point, is not supported by the present findings.

According to Bayliss, the free acid of Congo red forms blue adsorption compounds with aluminum hydroxide, thorium hydroxide, leucine, and silk. When any one of these compounds is heated, the color changes to a red. Bayliss believes that this change of color follows upon a chemical reaction with the formation of a salt; and that adsorption is a preliminary to chemical combination. In this connection, the writer has prepared a red Congo fibrin, the minute particles of which turn blue in an acid solution. If, after sedimentation, the supernatant acid be drawn off, and boiling distilled water be poured on the blue particles, the latter turn red practically instantly. These manipulations can be carried out repeatedly with the same particles without producing any separation of dye. Although it is conceivable that an adsorption compound can be transformed into a true chemical union, it is difficult to see how a chemical compound can, by a single, simple manipulation, be changed back into the original adsorption complex.

In the present work an intimation of the real nature of dyed proteins has been furnished. If a protein is aggregated with a dye, acid or basic, which is readily dialyzable (ponceau, methylene blue), the dye can be virtually completely washed away from the protein with hot water. If, however, the dye does not dialyze (Congo red, night blue), aggregates with proteins can be made which do not yield any dye to boiling water. The conclusion of the writer is that these dyed proteins are not adsorption compounds in the ordinary sense, but that they consist of inclusions of the dye within innumerable minute ultrafilters formed during the coagulation of the protein.

THE APPLICATION OF G. BREIT'S FORMULA FOR CONDUCTANCE THROUGH HETEROGENEOUS MEDIA TO BLOOD.

By J. F. McCLENDON.

(From the Laboratory of Physiological Chemistry, University of Minnesota Medical School, Minneapolis.)

Much work on the electric conductance of blood is confused by the use of alternating currents and failure to take into account the capacity resistance of the cells. The use of direct currents obviates this confusion. The conductance of corpuscles, serum, and various mixtures of serum and corpuscles was measured and G. Breit's formula used to calculate the conductance of the cells.[28]

Calculations from Ohm's law could not be made accurately. A Wheatstone bridge using calomel electrodes separated from the blood by stop-cocks, filled with agar-gel, gave reproducible results if the current was reversed four times a minute. The resistance of the electrodes was subtracted from the total. Mixtures of corpuscles and serum were made by weighing and dividing by specific gravity. The conductivity vessel was immersed in an oil bath.

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[28] Breit, G., *Koninklijke Akad. wetensch Amst.*, 1922, xxv, 293.

DISTRIBUTION, DESTRUCTION, AND EXCRETION OF URIC ACID.

By OTTO FOLIN, HILDING BERGLUND, and C. DERRICK.

ON CERTAIN METHODS EMPLOYED IN THE STANDARDIZATION OF INSULIN.

By GEORGE B. WALDEN and G. H. A. CLOWES.

CHEMICAL CHANGES IN THE EXPERIMENTAL ATROPHY OF MUSCLE.

By K. K. CHEN and H. C. BRADLEY.

ANTIKETOGENESIS: THE IN VITRO KETOLYTIC REACTION.

By P. A. SHAFFER and T. E. FRIEDMANN.

ON FURTHER OBSERVATIONS REGARDING THE INFLUENCE OF pH RANGE ON FERTILIZATION AND CELL DIVISION IN MARINE EGGS.

By HOMER W. SMITH and G. H. A. CLOWES.

THE LOCALIZATION OF THE SECRETION OF ACID IN THE KIDNEY.

By A. B. MACALLUM.

A NEW THEORY OF URINARY SECRETION.

By A. B. MACALLUM.

PHYSIOLOGICAL RESPONSE TO EXERCISE.

By ETHEL RONZONI.

ALKATAN.

By J. S. HEPBURN and R. H. STROH.

ABSTRACT OF THE EXECUTIVE PROCEEDINGS OF THE EIGHTEENTH ANNUAL MEETING.

New Members: C. H. Best, Meyer Bodansky, A. P. Briggs, G. L. Foster, Mary L. Foster, W. H. Griffith, E. G. Gross, A. R. Lamb, T. P. Nash, Jr., J. M. Nelson, Ethel Ronzoni, Elmer Severinghaus, G. E. Simpson, Barnett Sure, H. B. Vickery.

Clinical Members: Hilding Berglund, W. S. McCann.

Resignation Accepted: A. E. Austin.

Deceased Members: Frank Cummings Cook, Jacob Rosenbloom.

Officers for the Year 1924: *President*, Philip A. Shaffer; *Vice-President*, Henry C. Sherman; *Secretary*, D. Wright Wilson; *Treasurer*, Walter R. Bloor; *Additional Members of the Council*, Otto Folin, Andrew Hunter, Victor C. Myers; *Nominating Committee*, S. R. Benedict, H. C. Bradley, E. C. Kendall, H. B. Lewis, E. V. McCollum, A. P. Mathews, L. B. Mendel, C. P. Sherwin, D. D. Van Slyke.

Editorial Committee 1924–1929: L. J. Henderson, Walter Jones, Graham Lusk.

Dues: The dues for the year 1924 were fixed at $2.00.

Attendance: Sixty-eight members of the Society attended the meeting.

Victor C. Myers,
Secretary.

PROCEEDINGS

OF THE

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

NINETEENTH ANNUAL MEETING
Washington, D. C.

December 29-31, 1924

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ABSTRACT OF EXECUTIVE PROCEEDINGS

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Abstracts of Scientific Proceedings 3
Abstract of Executive Proceedings 67

For the List of Members and the Constitution and the By-Laws of the Society see the 1925 Register of the Federation of American Societies for Experimental Biology, comprising the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, and the American Society for Experimental Pathology.

Reprinted from The Journal of Biological Chemistry
Vol. LXIII, pp. xi-lxxiv, 1925 (February)

SOME APPLICATIONS OF THE NEW CYSTEINE REACTION.

By M. X. SULLIVAN.

(From the Division of Chemistry, Hygienic Laboratory, United States Public Health Service, Washington.)

The cysteine reaction—a red color with β-naphthoquinone-4-sodium sulfonate in the presence of alkali and a reducing agent such as Na_2SO_3—can be used to determine cysteine quantitatively in the presence of other amino acids and sulfydryl compounds. Cystine slowly gives the reaction to a slight degree. If cystine is treated with aqueous NaCN it is reduced to cysteine and then gives the reaction promptly. In strong polyneuritis on a rice diet, the tissues of pigeons have little if any cysteine, but do contain cystine; while on a rice diet plus vitamin, the tissues of the pigeons contain cysteine as well as cystine. In extreme polyneuritis the reducing capacity of the tissues seems to be suspended.

THE QUANTITATIVE STUDY OF THE PHYSIOLOGIC ACTION OF THYROXIN.

By EDWARD C. KENDALL.

(From the Department of Biochemistry, Mayo Foundation, Rochester, Minnesota.)

The isolation of thyroxin in pure crystalline form permitted the fact to be shown that thyroxin alone increases the rate of combustion in the animal organism. Furthermore, this increase is related quantitatively to the amount injected. 1 mg. given to an adult produces an increase of approximately 2.5 per cent. The substance acts in minute amounts for long periods, and produces such enormous increases in the output of carbon dioxide above the former level, that there is no escape from the conclusion that thyroxin acts as a catalyst.

Through a study of closely related compounds, which are synthetically prepared, the fact was demonstrated that thyroxin can exist in two forms: reduced and oxidized. Thyroxin, as isolated from the gland, is the reduced form.

Alpha-oxy-indole propionic acid, the precursor of thyroxin, acts as a reducing agent. It loses 2 atoms of hydrogen with molecular oxygen when the pyrrole ring in the molecule is open, and forms a bond from the nitrogen to number seven carbon in the benzene ring. This compound has feeble oxidizing power. When, however, the pyrrole ring is closed and the bond is present from the nitrogen to number seven carbon, the oxidizing power of the compound is very much increased. The oxidizing potentials of the open and closed ring compounds, when both exist in their oxidizing form, have been measured, and a difference of at least 0.3 volt was found.

When they are injected into a normal dog, the reduced form and the oxidized but open ring form produce no visible response; the oxidized closed ring form causes a marked physiologic effect. There is a drop in blood pressure, an increase in pulse rate, marked increase in respiration, and an increase in the rate of metabolism.

The function of thyroxin is to furnish a compound that can be acted on by mild oxidizing agents, among which is molecular oxygen, and which can then by an intramolecular rearrangement produce an intensely oxidizing substance.

It is significant that oxidation in the animal organism is accelerated by the presence of an agent which is an active hydrogen acceptor, and the degree of stimulation is dependent on the oxidizing potential of this hydrogen acceptor.

A METHOD FOR THE DETERMINATION OF THE pH OF CEREBROSPINAL FLUID.

By A. T. SHOHL and IRVINE McQUARRIE.

(From the Department of Pediatrics, Yale University, New Haven.)

The spinal fluid is obtained over mercury without exposure to air. The sampling tube is calibrated at 0.1 cc. for the first cc. and at 1 cc. intervals up to 10 cc. A measured amount of phenol red is introduced. The lumbar puncture needle is connected to the receiving tube by a sterilized glass connecting piece and with a short rubber tube. The technique is similar to that used in obtaining blood for measuring the CO_2 content. The sampling tube is of the same diameter and same thickness as the comparator tubes. Without transfer the pH is read in the sampling tube colorimetrically at 38° by comparing either with Sörensen's phosphate standards at 38° and subtracting 0.03 pH or by means of the bicolorimetric standards of Hastings and Sendroy. The CO_2 content is determined by Van Slyke's method. Using a pK value of 6.20 as determined experimentally and from the literature the tension of CO_2 can be calculated.

DISTRIBUTION OF ELECTROLYTES IN BLOOD.

By D. D. VAN SLYKE and A. B. HASTINGS.

(From the Hospital of The Rockefeller Institute for Medical Research.)

The conclusion of Van Slyke, Wu, and McLean[1] has been confirmed that HCO_3 in blood is distributed between cells and serum in such manner that the ratio $[HCO_3]_{cells} : [HCO_3]_{serum}$ varies with CO_2 tension in agreement with a formula deduced from Donnan's law and the base-binding powers of the cell and serum proteins, the validity of the laws of ideal solutions being assumed as a first approximation. The effect of oxygen tension changes has now been also studied, and found to agree with that predicted from the difference in base-binding power between reduced and oxygenated hemoglobin. The ratio $[Cl]_{cells} : [Cl]_{serum}$ has likewise been studied, using the nitric acid ashing method for Cl determination.[2] The absolute values for the Cl ratios are somewhat lower than the HCO_3 ratios, but parallel the latter and show approximately the same changes with varying pH and oxygenation. In order to obtain direct data on ion activity ratios, the ratios $[H^+]_{cells} : [H^+]_{serum}$ and $[Cl']_{cells} : [Cl']_{serum}$ were determined electrometrically. The ratios thus determined were found to approach equality, to lie at a lower level than the total [Cl] and $[HCO_3]$ ratios, but to parallel the latter as influenced by pH and oxygen changes.

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[1] Van Slyke, D. D., Wu, H., and McLean, F. C., *J. Biol. Chem.*, 1923, lvi, 765.

[2] Van Slyke, D. D., *J. Biol. Chem.*, 1923–24, lviii, 523.

MOVEMENT OF ELECTRICALLY CHARGED ATOMS INSIDE RED BLOOD CORPUSCLES.

By J. F. McCLENDON.

(From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis.)

Ionic mobility was measured by means of the Wheatstone bridge method for determining electric conductivity of solutions. In order to reduce inductance the resistance wires were perfectly straight and the resistance of the cell, full of erythrocytes washed in sugar solution, was balanced against the resistance of a similar cell filled with a known KCl solution at 25°. Owing to the insulating properties of the surfaces of the erythrocytes, the resistance, R, to direct current is very high. The impedance, Z, to alternating current of low frequency was also high since $Z = \sqrt{X^2 + R^2}$ where X is the reactance due to the insulating membranes and $X = \frac{1}{2\pi f C}$ where f is the frequency and C is the capacitance of the condensers formed by interposition of the insulating membranes. By increasing the frequency the reactance is reduced and when this is reduced to a negligible quantity, the resistance of the cell interiors may be determined. The only difficulty lies in the fact that the volume occupied by the membranes and the sugar solution is unknown. The high resistance of the membranes is illustrated by the fact that if we take the impedance measured with a frequency of 1,000,000 cycles per second as the resistance of the erythrocyte interior, the impedance is increased 400 per cent due to the reactance of the membranes when measured with a frequency of 1,000 cycles per second. Preliminary experiments indicate that the conductivity of the ox erythrocyte interior is about equal to or greater than that of a 0.01 N KCl solution.

PHYSICOCHEMICAL METHODS OF CHARACTERIZING PROTEINS.

VII. THE MOLECULAR WEIGHTS OF THE PROTEINS.

By EDWIN J. COHN.

(*From the Department of Physical Chemistry in the Laboratories of Physiology, Harvard Medical School, Boston.*)

The molecular weights of the proteins constitute their outstanding physical characteristic. Their estimation by such direct methods as the determination of the osmotic pressures they produce has, however, not often[3] led to consistent results.

Although the molecular weights of most proteins have been in doubt, the minimal molecular weight of hemoglobin has been well known for over 30 years. In 1894 Hüfner found that 16,721 gm. of hemoglobin combined with each mol of carbon monoxide, or with a weight of the protein that contained 1 atom of iron. The simultaneous consideration of analytical and physicochemical information thus led, at an early date, to a very accurate minimal molecular weight for hemoglobin and has subsequently led to estimates of the minimal molecular weights of other proteins.

Solubility and electromotive force measurements offer new physicochemical methods of determining the equivalent combining weights of proteins, and amino acid determinations in protein hydrolysates provide new analytical evidence from which can be calculated the minimal weight of a protein that contains 1 molecule of an amino acid. From such analytical and physicochemical data the minimal molecular weights of fourteen proteins have been deduced.

Given the minimal molecular weights of proteins, their true molecular weights may be estimated by determining the relative size of their molecules. This is being accomplished by dialysis and ultrafiltration through membranes of graded permeability. The probable molecular weights of the proteins are thus obtained (Table I) as integral multiples of their minimal molecular weights.

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[3] The investigations of Sörensen, in which account has been taken of membrane equilibria, constitute the outstanding exceptions. His results are in good agreement with those reported.

TABLE I.

Protein.	Minimal molecular weight.	Probable molecular weight.
Bence-Jones' protein	12,250	24,500
Egg albumin	33,400	33,400
Serum albumin	5,000	45,000
Hemoglobin, horse	16,700	66,800
" ox	33,400	66,800
Fibrin.	14,000	42,000*
Serum globulin	27,000	81,000*
Hemocyanin, *Limulus*	22,700	90,800*
Zein	19,400	97,000*
Glutenin	36,300	108,900*
Edestin	29,000	116,000*
Gelatin	10,300	123,600*
Gliadin	20,700	125,000*
Casein	12,800	192,000*

* The estimation of the molecular dimensions of these larger proteins is not yet complete. The true molecular weights may, therefore, be still larger multiples of these minimal molecular weights.

ACETYLATION AS A DETOXICATING REACTION.

By JOSEPH B. MUENZEN, LEOPOLD R. CERECEDO, AND CARL P. SHERWIN.

(*From the Chemical Research Laboratory, Fordham University, New York.*)

In studying the detoxication of para-aminophenylacetic acid it was found that the human being as well as the rabbit detoxicates the compound by joining acetic acid to the amino group, while the dog detoxicates the foreign molecule by conjugating glycocoll with it, thus forming para-aminophenaceturic acid. A number of amino compounds were investigated in order to determine the influence of various other radicals on acetylation. It was found, contrary to the report of Salkowski, that the human organism is able to acetylate both para- and meta-aminobenzoic acid.

In general we found that the dog will acetylate an aliphatic amino compound or the side chain of an aromatic compound, while the human being and the rabbit may fail to acetylate these compounds entirely, but are able to acetylate the amino groups attached directly to the benzene ring. Acetylation in the body is apparently limited to amino compounds.

The influence of diet on acetylation was studied, as well as the effect of certain amino acids and certain compounds believed to be products of intermediary catabolism of proteins, fats, and carbohydrates.

Attempts were made to locate the seat of the reaction, and it was found that acetylations in the human being as well as in the rabbit are very likely confined to the liver.

The possibility of using this reaction as a functional liver test was investigated and yielded promising results.

THE RELATION OF HISTIDINE AND ARGININE TO CREATINE AND PURINE METABOLISM.

By WILLIAM C. ROSE and KENNETH G. COOK.

(From the Laboratory of Physiological Chemistry, University of Illinois, Urbana.)

Studies have been made of creatine and purine metabolism in growing rats upon diets in which the nitrogen was supplied, respectively, in the form of casein, completely hydrolyzed casein, and hydrolyzed casein from which histidine and arginine had been precipitated by the Kossel-Kutscher procedure. Despite the inherent difficulties involved in quantitative urine collections in rats, the results of the experiments are quite consistent. The diets of whole casein and of completely hydrolyzed casein led to the excretion of progressively increasing quantities of total creatinine (creatine plus creatinine), allantoin, and uric acid, which were roughly proportional to the increments in body weight of the animals. On diets of hydrolyzed casein from which histidine and arginine had been precipitated, the output of allantoin decreased 40 to 50 per cent. The uric acid excretion also showed a tendency to decrease, but the variations from the normal values were less striking than in the case of allantoin. Total creatinine generally manifested a moderate increase followed by a decline, but in no case did the elimination on the inadequate diet fall below the output on the adequate ration.

That the above effects are not to be attributed solely to the losses in weight of the rats on the deficient diets is indicated by the results of experiments in which a deficiency of tryptophane was induced. When the ration was adequate in every respect except as regards its tryptophane content, the animals steadily declined in weight, but the output of allantoin and uric acid remained quite constant.

The addition of histidine to diets in which the component hydrolyzed casein had previously been subjected to silver precipitation led to increases in the excretion of total creatinine, uric acid, and allantoin, until the quantities eliminated were of the same order as in animals upon whole casein. On the contrary, the addition of arginine to the deficient rations failed entirely to affect the output of any of the urinary components. It is evident from these investigations, as well as from the growth experiments already described,[4] that arginine and histidine are not interchangeable in metabolism. But as far as the relation of *histidine* to purine metabolism is concerned, our data confirm the findings of Ackroyd and Hopkins,[5] and indicate that *under ordinary conditions of diet this amino acid is probably the mother substance of allantoin.*

Under the conditions which pertained in our experiments, *no relationship was observed between the arginine content of the of the diet and the total creatinine elimination in the urine.* As stated elsewhere,[4] it is quite likely that the Kossel-Kutscher method of precipitation does not completely remove arginine. Assuming, therefore, that arginine is the precursor of creatine, it is possible that on the deficient diets our animals may have received adequate amounts of this amino acid for creatine anabolism, and that quantities fed in excess of the anabolic needs were catabolized by paths other than that which leads to creatine-creatinine production.

URIC ACID FORMATION IN THE CRUSTACEAN, PANULIRUS ARGUS.

By SERGIUS MORGULIS.

(*From the Department of Biochemistry, University of Nebraska, Omaha.*)

The blood of the crawfish *Panulirus* becomes entirely free of uric acid when it has been kept for a day or two without food. It has been found that if urea (1 to 3 gm.) is then injected intramuscularly the uric acid reaction invariably reappears in the blood filtrate, the amount of uric acid frequently being in sufficient amount for quantitative determination. Administering the urea by way of the intestinal canal does not produce this effect. Likewise, experiments where various ammonium salts were used for the injection either failed to reproduce the results obtained with the urea or, as in the case of small doses of ammonium lactate, this result was obtained in a slight degree. Experiments performed with glycine, however, frequently did yield positive results, though in no instance were the effects as definite as in the case of urea injections. The blood uric acid was determined both by the Benedict direct method and by the method of Morris-Macleod, where the uric acid is precipitated preliminary to the development of the color. The results were essentially the same by both procedures. Space limitations do not permit a discussion of the reasons for the hypothesis that the uric acid is synthesized under the conditions of these experiments.

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[4] Rose, W. C., and Cox, G. J., *J. Biol. Chem.*, 1924, lxi, 747.

[5] Ackroyd, H., and Hopkins, F. G., *Biochem. J.*, 1916, x, 551.

THE SYNTHESIS AND EXCRETION OF HIPPURIC ACID BY RABBITS.

By WENDELL H. GRIFFITH.

(From the Department of Biological Chemistry, St. Louis University, St. Louis.)

The rate of excretion of combined benzoic acid in the urine in the 6 hour period after the administration of sodium benzoate was determined for rabbits on a normal diet and on a diet to which thyroid powder was added. The addition of the thyroid powder (0.2 gm. of Armour's desiccated thyroids per day) to the diet resulted in an increased output of combined benzoic acid in the experimental period. This increased rate of excretion of combined benzoic acid might have been due to: (1) an increased rate of synthesis of glycine, or (2) to the presence of extra preformed glycine resulting from the breakdown of tissue protein, or (3) to an increased excretion of other forms of combined benzoic acid. A study of the nitrogen of the urine showed an increased total nitrogen and a marked creatinuria, but very little, if any, change in the hippuric acid nitrogen. The increased output of combined benzoic acid was considered to be due to the presence of other forms of combined benzoic acid rather than to an increased synthesis and excretion of hippuric acid.

THE EFFECT OF REPEATED ADMINISTRATION OF SMALL AMOUNTS OF CYSTINE ON THE RABBIT.

By HOWARD B. LEWIS.

(*From the Laboratory of Physiological Chemistry, University of Michigan, Ann Arbor.*)

Cystine has been administered orally as the sodium salt in small doses (0.50 to 1.0 gm. per kilo) for successive days to fasting rabbits and to rabbits on a diet of oats and cabbage. After the 1st day protein and casts appeared in the urine, the excretion of total non-protein nitrogen and creatinine was depressed, but no marked increases in the amino acid and cystine excretion were observed. The blood showed a marked rise in the non-protein nitrogen and a slight increase in the amino nitrogen as determined by the Folin method.

THE OPTICAL ACTIVITY OF CYSTINE.

By JAMES C. ANDREWS.

(*From the Department of Physiological Chemistry, University of Pennsylvania, Philadelphia.*)

The values previously recorded in the literature for the specific rotation of cystine in acid solution vary from −200° to −250°. It was found necessary to determine whether this variation was due to the conditions of the determinations or to the racemization which takes place under strongly acid conditions. In the present work variations in the kind of acid employed, its excess concentration, concentration of cystine, and concentration of other electrolytes present were studied. The results showed that proper variation of the above conditions can produce, in a single sample, specific rotation values ranging from −190° to −270°. Although for a given acid, the effect of change in pH and in cystine concentration was usually the same, several as yet unexplained anomalies were encountered and the effect of individual ions was found to be highly specific, particularly when used in the form of salts. An extension of Patterson's theory of the effect of internal pressure on the form of asymmetric molecules in solution is advanced to account for the effects observed. In the present work the changes in internal solution pressure are produced by the varying degrees of hydration of the different ions present.

THE REACTION OF ACETOACETIC ACID WITH THE HEXOSES.

By THEODORE E. FRIEDEMANN.

(*From the Laboratory of Biological Chemistry, Washington University, St. Louis.*)

A further study was made of the ketolytic reaction described by Shaffer[6] and by Shaffer and Friedemann,[7] which is very similar to, and perhaps the basis for the phenomenon of antiketogenesis. Under optimum conditions in the presence of peroxide, alkali, and glucose, approximately 2 mols of acetoacetate are oxidized for each mol of glucose. Similar ratios are obtained with fructose, mannose, and glycol aldehyde.

In the presence of peroxide, *the rate of acetoacetate consumption and oxidation to acids, parallels the rate of oxidation of glucose.* This suggests that the active ketolytic derivative of the sugar is some oxidation product whose rate of condensation with acetoacetate is greater than its rate of further oxidation. However, one of the first oxidation products of glucose, glucosone, is not ketolytic in alkaline peroxide solutions, although it is ketolytically active in neutral or slightly alkaline solutions in the absence of H_2O_2. In this respect it behaves similar to glyoxal, the oxidation product of glycol aldehyde.

This may indicate as interpreted by us in the case of the ketolytic reaction of glycol aldehyde that the condensation is not with an oxidation product but with a form of glucose, *before* oxidation. This is possible, since in high concentration glucose and acetoacetate do react, the rate paralleling the acid formation, most of which is CO_2.

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[6] Shaffer, P. A., *J. Biol. Chem.*, 1921, xlvii, 433.

[7] Shaffer, P. A., and Friedemann, T. E., *J. Biol. Chem.*, 1924, lxi, 585.

THE RATE OF ELIMINATION OF VARIOUS INGESTED MONOSACCHARIDES IN PHLORHIZIN DIABETES.

By H. J. DEUEL, Jr., and W. H. CHAMBERS.

(From the Department of Physiology, Cornell University Medical College, New York City.)

The hourly rate of elimination of "extra" glucose in the urine of dogs rendered diabetic with phlorhizin has been studied for 12 hour periods after the oral administration of glucose, galactose, fructose, and lactose in 16 gm. doses. The rates of excretion of glucose and galactose were practically identical with that of glucose itself, thus indicating that the intermediary transformations involved in the change of these monosaccharides to glucose is a very rapid one. The "extra" glucose recovered after glucose ingestion was 106 per cent of the theoretical in two experiments, 93 and 94 per cent after fructose and 88 per cent after galactose. With lactose, a 50 per cent recovery of glucose in 12 hours was obtained, but the hourly rate of excretion was slower and more constant.

A sparing action on the nitrogen metabolism was noted in each case despite the fact that none of the sugar was burned. The decreased nitrogen metabolism became evident the 2nd hour after the feeding of the carbohydrate and reached the minimum value 6 to 11 hours thereafter. The nitrogen level remained at this low value for several hours and then the amount eliminated gradually increased. It was noted that the mere ingestion of glucose by the fasting, phlorhizinized dog restored its muscular power even though the glucose was quantitatively excreted in the urine.

ON THE NATURE OF URINARY GLUCOSE.

By W. C. AUSTIN and T. E. BOYD.

(From the Laboratories of Physiological Chemistry and Physiology, Loyola University, Chicago.)

Female dogs were fasted and phlorhizinized. Fresh urine specimens were catheterized, clarified, and preserved. Comparisons of polariscopic measurements over long periods of time showed constant rotation, which coincided very closely with copper reduction values for glucose content as estimated by the Shaffer-Hartmann method. Injections of insulin did not alter the ratios of polariscopic to copper reduction values. The conclusion is drawn that there is no mutarotatory ethylene oxide form of glucose in urine of phlorhizinized dogs, even under the influence of insulin. Is the nature of such urinary glucose the same as that of blood sugar?

ADEQUACY OF PIGEONS AND RATS FOR VITAMIN B STUDIES.

By A. D. EMMETT and GAIL E. PEACOCK.

(*From the Medical Research Laboratories, Parke, Davis and Company, Detroit.*)

Data have been accumulated over a considerable period of time on the vitamin B requirements of pigeons and rats. Our experiments show that the pigeon, judged by the weight curve, as well as the prevention of polyneuritis, can be used to determine quantitatively the vitamin B, with as fair a degree of accuracy as the rat. Whether the basal diet of the pigeon is polished rice, or one high or low in carbohydrates, or the same as the regular vitamin B deficient ration generally given rats, does not seem to alter the conditions to any essential degree. When given a basal diet of polished rice supplemented with 0.00015 gm. of one of our concentrates, pigeons show a decided gain in weight, whereas, when the dose is reduced to 0.0001 gm., the birds are kept in equilibrium, maintaining their weight. The vitamin B maintenance requirements of mature pigeons always runs two to three times higher than the normal growth requirements of young rats.

QUANTITATIVE ASPECTS OF THE FUNCTION OF VITAMIN B IN SEVERAL SPECIES.

By GEORGE R. COWGILL, ARTHUR H. SMITH, and H. H. BEARD.

(*From the Laboratory of Physiological Chemistry, Yale University, New Haven.*)

Data obtained from three widely different species of animals —mouse, rat, and dog—indicate that the vitamin B requirement is closely related to metabolism.

In the *adult* animal the vitamin B requirement is very closely proportional to the product of: (1) the two-thirds power of the weight—which may indicate surface or "active protoplasmic mass" or whatever the metabolism depends on, (2) the quantity of energy handled as indicated by the total calories utilized from the food, and (3) the body weight. The following formula has been constructed embracing these factors. By it a number called provisionally the "vitamin B constant" may be calculated.

$$\frac{\text{Vitamin B}_{per\ day}}{\text{Weight}^{\frac{2}{3}}} = K_{vitamin} \times \text{calories}_{per\ day} \times \text{weight} \quad (a)$$

whence

$$K_{vitamin} = \frac{\text{vitamin}_{per\ day}}{\text{calories}_{per\ day} \cdot \text{weight}^{\frac{2}{3}} \cdot \text{weight}} \quad (b)$$

$$K_{vitamin} = \frac{\text{vitamin}}{\text{calories} \cdot \text{weight}^{\frac{5}{3}}} \quad (c)$$

This formula gives good agreement with experimental data for *adult* rats, dogs, and mice. During *growth* some other factor, as yet undefined, appears to affect the vitamin requirement, $K_{vitamin}$ during this period, having a higher value. Complete analysis of the growth phenomena may disclose this factor and enable a correction to be applied to this formula giving it more widespread significance.

The precise value of the vitamin "constant" just described is peculiar (1) to the species, the value being greatest with the mouse, and smallest with the dog, (2) to the source of vitamin B used in the test, and (3) to the particular set of conditions maintained in the investigation. Under any given set of experimental conditions the value is constant within the limits of error of the accepted methods of vitamin testing.

In view of the wide variation in growth rate and food intake shown by rats and mice, particularly when the vitamin B supply is close to the minimum, statistical treatment of the data becomes necessary. This requires that voluminous data be secured in studies of this nature.

ANTIRACHITIC ACTIVATION BY LIGHT.

BY H. STEENBOCK, A. BLACK, E. M. NELSON, M. T. NELSON, AND C. A. HOPPERT.

(*From the Department of Agricultural Chemistry, University of Wisconsin, Madison.*)

Using two methods of testing for the presence of antirachitic activation; namely, (*a*) the induction of growth and calcification in the rat during phosphate inhibition and (*b*) the cure of rickets produced by deficient phosphate and excess of calcium in the diet, it has been found that a large number of naturally occurring materials can be activated antirachitically by exposure to ultraviolet light. Among these are yeast, commercial casein, ox bile, lanolin, and grains and their commercial products such as starch, meals, flours, breakfast foods, and oils.

The antirachitic activation apparently cannot be induced in pure protein, carbohydrate, or fat or such substances as salts, water, ether, hydrochinon, phloroglucin, or paraffin hydrocarbons. It appears to be localized in the unsaponifiable lipoidal constituents as the sterols and closely related compounds. Cholesterol purified first by numerous crystallizations and then as a benzoate and finally as an acetate can be activated. Phytosterols cannot always be activated. Digitonin will not precipitate all the active constituents from the unsaponifiable fraction of cod liver oil.

It follows: (*a*) that a definite function for cholesterol has been discovered; and (*b*) that irradiation of naturally occurring or synthetic compounds is available as a new principle in the pharmacotherapy of diseases known to respond to direct irradiation.

A FURTHER REPORT ON IMPARTING ANTIRACHITIC PROPERTIES TO INACTIVE SUBSTANCES BY ULTRA-VIOLET IRRADIATION.

BY ALFRED F. HESS AND MILDRED WEINSTOCK.

(*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.*)

Activity has been induced in linseed or cottonseed oil by irradiation with the mercury vapor lamp and was found to be practically unimpaired in tests carried out 6 months later. The oil likewise acquired antirachitic properties when irradiated in an atmosphere of nitrogen. As in cod liver oil the specific principle of the activated oil was found to be present in the non-saponifiable fraction; it was absent in the non-saponifiable fraction of ordinary non-irradiated oil. Glycerol, gelatin, and milk (in the small doses fed) were not rendered antirachitic by irradiation. Wheat, whether green or etiolated, was endowed with the antirachitic property by means of irradiation and maintained this property for a period of at least 2 weeks. The same is true in regard to green or yellow lettuce which, under ordinary conditions, has no protective value. Potency is retained in these vegetables at least for several days. Refined wheat flour can be rendered active by this means. Cholesterol, as well as phytosterol, was found to have no antirachitic virtue when fed to rats, but after they had been exposed to irradiation, parallel tests showed that they had acquired this property.

DIETARY REQUIREMENTS FOR REPRODUCTION.

IV. POSITIVE EVIDENCE FOR THE EXISTENCE OF A REPRODUCTIVE DIETARY COMPLEX (VITAMIN E) SOLUBLE IN ETHER, BENZENE, AND ACETONE.

By BARNETT SURE.

(*From the Department of Agricultural Chemistry, Agricultural Experiment Station, University of Arkansas, Fayetteville.*)

No fertility was secured with the control ration having the following composition.

	per cent
Skimmed milk powder	50 0
Ferric citrate	0.2
Agar-agar	2.0
Harris yeast-vitamin powder (later increased to 1.0 per cent)	0.5
Cod liver oil	2.0
Dextrin	45.3

When 5 per cent of dextrin was replaced by 5 per cent of either ether, acetone, or benzene extracts of the wheat embryo fertility was always obtained, but, in order to secure absolute success in lactation, the Harris yeast-vitamin powder, which was used as a source of water-soluble B vitamin, had to be increased to 1.0

per cent of the total ration. Healthy second generations are growing up normally on skimmed milk powder rations containing 3 and 5 per cent of the wheat germ oil.

Wheat embryo extracted with ether, acetone, or benzene produces fertility (on account of incomplete extraction of all the fat as shown by chemical analysis), but is inadequate for lactation. The addition of acetone or ether extracts of the wheat embryo to wheat germ preparations extracted with such organic solvents results in success not only in fertility but also in rearing of young.

The evidence presented clearly shows the existence of a specific dietary complex essential for reproduction which is soluble in organic solvents, and it is proposed that this vitamin be termed "fat-soluble E."

THE INFLUENCE OF MILK RATIONS HIGH AND LOW IN FATS ON THE SEX GLANDS OF MALE ALBINO RATS, WITH SPECIAL REFERENCE TO SUBSTANCE X.

By H. A. MATTILL and M. M. CLAYTON.

(*From the Department of Vital Economics, The University of Rochester, Rochester, New York.*)

The prevention of sterility in male rats on milk rations by substance X or by lowering the fat content of the ration is confirmed. Table I summarizes the data on gonad weights secured from some of the male animals examined after 140 days of age.

TABLE I.

	Total No. of animals.	Those having normal gonads.	Those having gonads less than 80 per cent of normal weight.
High fat ration	41	7	34 (83 per cent)
" " " plus X* 1st generation	10	7	3 (30 " ")
" " " " " 2nd "	15	14	1 (6 " ")
Low fat ration	16	10	6 (38 " ")
" " " 2nd generation	5	0	5 (100 " ")
Stock colony animals	38	35	3 (8 " ")

* Unextracted wheat embryo or lettuce leaves.

These figures also indicate that on rations low in fat, X cannot be dispensed with. It must be supplied in high fat rations during adolescence and early maturity. Of fifteen animals transferred from inadequate to adequate diet after 130 days of age, only one failed to show degeneration. Adequate food for as long as 200 days thereafter failed to restore fertility. Conversely, of ten animals transferred from adequate to inadequate rations before 90 days of age, only one escaped degeneration. Of fourteen so transferred after 90 days of age only three suffered degeneration. Substance X therefore appears to be required for the establishment of normal structure and function of the testes, but thereafter it is less critically needed. Of the unsaponifiable constituents of wheat embryo oil those transformable into cholesterol by the animal probably do not include X for the blood cholesterol of animals showing degeneration tends to be higher than that of normal animals.

MINERAL METABOLISM OF ADULT MAN.

By GUY W. CLARK.

(*From the Departments of Biochemistry and Pharmacology, University of California, Berkeley.*)

The work reported in this paper represents only one phase of a group attack[8] being made to determine, if possible, the direct causative and contributory factors involved in pyorrhea alveolaris.

Since the literature does not reveal any detailed and complete experiments in the field of mineral metabolism it becomes necessary to study such changes in normal individuals before any attempt is made to study similar processes where pathological changes are in progress.

Four adult males, representing different ages and of different temperaments, served as experimental subjects. The experiments were carried on for 28 weeks, during which time five different diets were fed. Composite samples of each of the foods were collected. A weekly urine composite was obtained by taking one-tenth of each day's excretion. Feces were dried and made into weekly composites. From the analyses of these composite samples weekly balances are being prepared for the following elements: calcium, magnesium, sodium, potassium, chlorine, phosphorus, sulfur, and nitrogen.

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[8] This work, made possible by a grant from the Carnegie Corporation to the University of California, is being carried on by the California Stomatological Research Group.

Blood analyses, which included calcium, magnesium, sodium, potassium, chlorine, acid-soluble phosphorus, and carbon dioxide, were made twelve times during the 28 weeks.

Not knowing how changes in the character of a diet might affect the chemical composition of the saliva, weekly samples of "resting" saliva were analyzed for the following elements: calcium, magnesium, sodium, potassium, chlorine, sulfur, and acid-soluble phosphorus. Determinations of total solids, organic matter, ash, and carbon dioxide were also made.

A complete report of this work will be made in the near future.

CALCIUM AND PHOSPHORUS METABOLISM IN DAIRY COWS.

By EDWARD B. MEIGS and WILLIAM A. TURNER.

(From the Dairy Division Experiment Station, United States Department of Agriculture, Beltsville.)

The calcium, phosphorus, and nitrogen balances have been followed continuously in two milking cows for a period of 175 days. During the whole of this period the animals were fed liberal amounts of grain, to which were added various kinds of hay. During 3 weeks of the experiment the rations were supplemented with cod liver oil.

The kinds of hay used were timothy and two grades of alfalfa. One lot of the alfalfa used had been well cured with no exposure to rain and with only a short exposure to direct sunlight. The other lot had been allowed to lie in the field for a week, and had been rained on for nearly 2 days before being dried. The good alfalfa contained about 2.3 per cent calcium; the poor alfalfa about 2.0 per cent; the timothy, about 0.3 per cent; and the grain, about 0.15 per cent.

While fed the good alfalfa, the cows remained fairly close to calcium equilibrium. On the poor alfalfa and timothy, they lost calcium rapidly from their bodies. The losses on the poor alfalfa were somewhat more rapid than those on the timothy hay in spite of the much higher calcium content of the former. The phosphorus metabolism followed the calcium in a general way, but by no means exactly.

In the course of 133 days on poor alfalfa and timothy hay, the cow giving the larger amount of milk lost about 1,300 gm. of calcium from her body, or about 19 per cent of the total normal calcium content of a cow's body. She was still giving 10 kilos of milk daily at the end of this period, and was losing calcium nearly as fast as she had at the beginning.

100 cc. of cod liver oil added daily during 21 days to the ration of grain and poor alfalfa hay did not improve the calcium assimilation in either cow, though it produced a noticeable increase in milk yield in one of them.

THE CALCIUM CONTENT OF THE BODY IN RELATION TO AGE, GROWTH, AND FOOD.

By H. C. SHERMAN and F. L. MacLEOD.

(*From the Department of Chemistry, Columbia University, New York.*)

The normal calcium content of the albino rat has been established by the analysis of large numbers of animals at definite ages from birth until full maturity. The percentage of calcium in the body averages about 0.25 per cent at birth and increases to from 1.0 to 1.2 per cent in the normal adult. Females which had not raised young contained a slightly higher average percentage of calcium than males of the same ages which had lived on the same diet and under the same conditions. The bearing and suckling of young diminished the calcium content of the mother's body. The effects of several differences in diet upon the calcium content of the body are described and discussed.

AGE CHANGES IN THE CHEMICAL COMPOSITION OF THE LONG BONES.

By FREDERICK S. HAMMETT.

(*From The Wistar Institute of Anatomy and Biology, Philadelphia.*)

In order to obtain an insight into the chemical processes concerned in bone formation during growth, and in order to provide standard data with which experimental alterations of bone composition, such as occur in rickets, can be compared, a determination was made of the water, organic matter, and ash; and the calcium, magnesium, and phosphorus content of the ash of the humerus and femur of normal male and female albino rats at the ages of 23, 30, 50, 65, 75, 100, and 150 days. The bones of ten rats of each sex of each age were analyzed.

It was found that the organic matter and ash percentage increase steadily with age, while the water percentage decreases. The curves of the ash increment and the water decrement are similar to that of an autocatalyzed monomolecular reaction. A slight disturbance in the course of ossification occurs in the females during puberty, which is not detectable in the males.

The percentage of ash and organic matter is greater in the bones of the females than in the males through the growth period observed. The water percentage is less in the females than than in the males.

The composition of the ash changes with age. The percentage of calcium increases. The percentage of magnesium and phosphorus tends to decrease. This holds for both bones in both sexes. A disturbance of calcification is suggested at puberty in both sexes. No consistent sex difference in percentage of magnesium or phosphorus is detectable during the growth period observed. The calcium percentage tends to be greater in both bones of the females, save at 30 and at 75 days of age. These are critical times in the physiological development of the albino rat as has already been shown from the refractometric studies of blood serum by Hatai.

STUDIES ON CHOLESTEROL.

II. INFLUENCE OF CHOLESTEROL ON NUTRITION AND GROWTH.

By ARTHUR KNUDSON and F. S. RANDLES.

(*From the Laboratory of Biological Chemistry, Union University Medical Department, Albany Medical College, Albany.*)

In a previous study we have demonstrated that cholesterol is synthesized in rats on a cholesterol-free diet. White rats were placed at the time of weaning on a cholesterol-free diet, but containing protein, carbohydrate, traces of fat, inorganic salts, and sources of vitamins A and B. On these diets good growth was obtained indicating that cholesterol is not essential for growth. To the cholesterol-free diets known amounts of cholesterol were added and there was no appreciable difference in their nutrition as evidenced by their rate of growth. The extracted cholesterol-free diet was deficient in the so called vitamin X of Evans, as evidenced by the fact that reproduction was very poor on this diet and that the number of litters born was considerably less than normal. Addition of cholesterol to this diet improves the fertility as shown by the fact that the number of litters born was greater and also that we have so far obtained a third generation while the second generation of rats on the cholesterol-free diet have failed to reproduce.

THE EFFECT OF WAKING ON URINARY CHLORIDES AND pH; SHORT INTERVAL URINES DURING THE SECOND DAY OF FASTING.

By GEORGE ERIC SIMPSON.

(*From the Department of Biochemistry, McGill University, Montreal, and the Department of Physiological Chemistry, University of Pennsylvania, Philadelphia.*)

If an experiment be so arranged that urine volume shows no marked change on waking in the morning after a night's sleep, it can be shown that hourly chloride excretion and urine pH definitely increase. When a subject stays awake all night and sleeps from 7 to 11 in the morning, urinary chlorides and pH decrease when the subject falls asleep, if the subject sleeps well at this abnormal hour. More often sleep is fitfull, and urinary chlorides and pH remain practically unchanged. Experiments are under way which it is hoped may determine if these results are to be explained on the basis of a salt shift from tissues or corpuscles to serum, and which is simultaneous with the demonstrated blowing off of CO_2 on waking.

Earlier work on short interval urines has been extended to include the 2nd day of fasting. The urine volume curve does not show the sharp variations exhibited during the 1st day of fasting. It is relatively smooth. Maxima for chlorides, phosphates, titratable acidity, and organic acids in the afternoon are about double the minimum level for these substances, found during the night. The curves show the same general contour, and resemble curves previously determined for phosphates by Fiske and by Broadhurst and Leathes. Such variations cannot be regarded as peculiar to phosphates.

CYCLIC VARIATIONS IN THE COMPOSITION OF FASTING BLOODS IN WOMEN.

By RUTH OKEY.

With the Cooperation of Statie E. Erikson, Ruth E. Boyden, Elda I. Robb, and Thelma Porter Levin.

(From the Laboratory of Household Science, University of California, Berkeley.)

The blood analyses here reported have been a part of a more general study of the variations in the metabolism of women in relation to the menstrual cycle. A group of twenty-five women students, normal in as far as could be determined by inspection of the records of the routine university physical examinations, have served as subjects. Three of these were on weighed and analyzed diets, constant for from 1 to 3 months at a time, for totals of 3, 5, and 6 months, respectively. The others were instructed to make as little variation as possible in their ordinary routines of diet and exercise while under observation. Blood samples were always taken before breakfast in the morning, and under as nearly uniform conditions as possible. The ordinary technique of the Folin-Wu system of analysis was followed unless otherwise noted.

Blood uric acid, as far as can be judged from a total of 280 determinations by the Benedict direct method, extending through forty monthly cycles, rises, just before or at the time of the onset of menstruation, to a level which averages 6 per cent above that for the intermenstrual period. This is followed, within from 1 to 3 days, by a fall to approximately 86 per cent of the average intermenstrual level for the individual. Within from 3 to 7 days after this fall, the uric acid rises to a level which is usually almost as high as that reached before menstruation. Following this latter rise, a decline to approximately the average intermenstrual level usually takes place within a few days. Extreme high and low values observed were 127 and 57 per cent, respectively, of the intermenstrual levels for the individuals concerned.

In approximately half of these blood filtrates, *uric acid* was also determined by the Morris-Macleod method. The curves so obtained showed the same type of premenstrual rise, menstrual fall, and postmenstrual rise, but they were not, in some cases, parallel to those based on the analyses by the other method. Data do not, however, justify at this time a discussion of the extent to which the occurrence of different forms of uric acid combinations in the blood may be responsible for these variations.

On a mixed diet which included purines, the menstrual variation in blood uric acid tended to be greater than that of the same individual on a purine-free diet. Moreover, while on long continued purine-free dietary regimes the pre- and postmenstrual rises in uric acid were still marked, the menstrual falls did not usually reach levels much below those of the average intermenstrual values. The number of cases on controlled diets is, however, too small to warrant any definite conclusions based on this observation. It is, moreover, unfair to assume that blood samples taken even as often as once a day will show the maximum effect of menstrual variations in any individual all the time.

Non-protein nitrogen values usually rose and then fell during menstruation, but these curves were neither so striking nor so uniform as those from the uric acid determinations. Only a part of the samples have been analyzed for *urea*, but these showed no evidence of a significant menstrual variation. Analyses of blood *creatine* and *creatinine* have, in general, confirmed the observations of Wang and Dentler[9] to the effect that there is no significant relationship between variations in the levels of these substances and the menstrual period.

The average values for fasting *blood sugars* tend to be slightly higher (1 to 8 mg. per 100 cc.) just before or during menstruation than at any other time. There is also a tendency, however, to larger deviations from the average values for blood sugar during this period. Hence, although a total of approximately 300 determinations has been made, any conclusion that there is a uniform variation in blood sugar level in relation to the menstrual cycle seems hardly justified by the data obtained. That the time of the menstrual period should, in as far as possible, be avoided in making blood sugar determinations for purposes of clinical diagnosis, does, however, seem to be indicated.

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[9] Wang, C. C., and Dentler, M. L., *J. Biol. Chem.*, 1920–21, xlv, 237.

A study of monthly variations in blood lipoids as determined by Bloor's revised methods of analysis has included approximately twenty-five cycles in fifteen subjects. A tendency to a rather abrupt fall in blood *cholesterol* (amounting, usually, to from 40 to over 100 mg. per 100 cc. of blood) during a period covering from 3 to 10 days before the beginning of the menstrual flow is indicated. After the onset of menstruation, the blood cholesterol begins to rise, usually somewhat abruptly at first then more slowly. Although there are day to day fluctuations of smaller magnitude, the general trend of the cholesterol curve usually continues to be upward until the period just preceding the next menstruation, when a fall again occurs. It is suggested that the time of this fall in blood cholesterol may be coincident with that of the most rapid growth of the corpus luteum, while the rise in the cholesterol level marks the period of its retrogression.

A rise in *fatty acid* was sometimes observed during or just preceding the fall in cholesterol, but this was by no means consistent for all cases. *Blood lecithin* showed little or no variation in relation to the menstrual period. Unfortunately, the amounts of the samples available have been too small to permit of separate analyses of plasma and corpuscles. A study of free cholesterol and cholesterol ester ratios is in progress.

PRELIMINARY REPORT ON THE BASAL METABOLISM OF 157 NORMAL SCHOOL CHILDREN BETWEEN THE AGES OF FIVE AND SEVENTEEN YEARS.

By IRENE SANDIFORD and ETHEL R. HARRINGTON.

(From the Section on Clinical Metabolism and the Section on Pediatrics, Mayo Clinic, Rochester, Minnesota.)

In order to establish with greater accuracy the standard basal metabolism of children we are carrying out metabolism studies on the school children of Rochester, Minnesota; thus far we have examined 79 girls (231 determinations) and 78 boys (224 determinations) quite evenly distributed between the ages of 5 and 17. The children were carefully selected by one of us (E. R. H.) and were in good health, although the routine determination of the oral temperature revealed a slight elevation of 1° in a small percentage of the tests. The children were very good and cooperated in practically all instances. They came to the laboratory at 7.15 a.m., without breakfast, and were allowed to rest half an hour before the metabolism determination was made. The gasometer method with analysis of the expired air was used according to the technique fully described elsewhere by Boothby and Sandiford. Single determinations were made on 3 successive days, except in a few cases in which the children came only once or twice.

The data, which have been plotted in various ways, will be presented in detail in the final paper. The results are expressed as calories for each square meter, using the Du Bois height-weight formula. As a rule the higher determination was obtained on the 1st day and generally the two subsequent determinations agreed more closely. A few of the higher determinations will be eliminated in drawing the average because of a slight elevation of the temperature or restlessness at the time of the determination. For comparison with the results of the other workers their averages are indicated on the chart by the various lines. As the study is still in progress we have thought it inadvisable at the present time to draw our own averages. However, it is obvious that our results for both boys and girls are falling between the upper triple line representing the Du Bois standards, and the lower triple line which indicates the Benedict-Talbot data. If only the lowest value of each one of the children is considered the general average for each year will be closer to the Benedict-Talbot than to the Du Bois line. However, the validity of omitting all but the lowest determination is open to question, when attempting to determine the best standard for clinical work. Besdale's results are, on the whole, lower than ours for both boys and girls, and this is also true of MacLeod's findings on girls of from 11 to 14. It is interesting to note that there is slightly less scattering of our data (not only of the single tests but also of the individual) among the girls than among the boys, probably owing to the fact that boys are more restless and more interested in watching the technical procedures than girls. The decrease in calories for each square meter progresses very regularly, and appears to be nearly, if not absolutely, a straight line function of age for both boys and girls from the ages of 5 to 17, and in this respect agrees more closely with the findings of Benedict and Talbot than with those of Du Bois.

THE INHIBITION OF AUTOLYSIS BY PROTEIN.

By A. B. HERTZMAN and H. C. BRADLEY.

(From the Department of Physiological Chemistry, University of Wisconsin, Madison.)

The addition of certain foreign proteins to an autolyzing liver brei inhibits the digestion of the liver, and the foreign protein itself is not digested. Egg albumin or the serum proteins give pronounced inhibitions of this sort. The inhibition is roughly proportional to the mass of foreign protein added, though never complete within the limits of our experiments. Other native proteins, such as edestin, produce no inhibition, and are themselves hydrolyzed.

The factor determining whether a foreign protein inhibits autolysis or not, is apparently the isoelectric point of the protein and its relation to the pH of the autolyzing liver. If the reaction of the liver brei approaches the pH value of the isoelectric point of the added protein, it does not inhibit and is itself digested. If, however, the isoelectric point of the foreign protein is more acid than the reaction of the brei, the protein inhibits autolysis.

We believe the explanation is that the protease distributes itself between the proteins present in the mixture and that the fraction fixed by the base-protein is in relatively stable combination. No hydrolysis results and no dissociation of the protease. The mixture thus has less active catalyst present than where no foreign protein was present. The autolytic decomposition of the liver, therefore, goes on more slowly than in the control, because there is less enzyme.

This, we believe, is additional evidence that in the normal living cell with its normal pH about 7 there is no active primary protease. It is present in stable base-protein-enzyme combina-

tion and can only be liberated by a rise of the H ion concentration.

The instability of the acid-protein-enzyme complex seems to be associated with a weakening of the peptide linkage such as might be assumed with a change in the valence of nitrogen in the imino group, from 3 to 5 with the addition of acid. The protein breaks hydrolytically at this point and the enzyme is also set free.

A METHOD OF OBTAINING FROM VEINS BLOOD SIMILAR TO ARTERIAL BLOOD IN GASEOUS CONTENT.

By SAMUEL GOLDSCHMIDT and ARTHUR B. LIGHT.

(*From the Department of Physiology, University of Pennsylvania, Philadelphia.*)

We have shown that blood taken from veins on the dorsal surface of the hand more nearly approximates arterial blood in its oxygen saturation and carbon dioxide content than does blood taken from veins in the antecubital space. In addition, when the hand and wrist are immersed in water at 45–47°C. and allowed to remain for 10 to 15 minutes, the oxygen saturation of the venous blood from the part is markedly increased.

Our results show that, because of the normally low oxygen unsaturation of blood from veins on the back of the hand, the application of heat, by increasing the blood flow through the hand, causes the arterial blood to pass into the veins with an indistinguishable change in its gaseous content beyond the limits of error of the methods of determination used.

Comparisons of blood, obtained under these conditions, from a vein on the dorsal surface of the hand, with blood from the brachial or radial artery have been made in six individuals. In nine other experiments, where only the venous blood was drawn, from veins on the back of the hand after exposure to hot water, figures of oxygen saturation within the range for arterial blood were obtained.

ON THE DETERMINATION OF NON-PROTEIN NITROGEN IN VERY SMALL AMOUNTS OF BLOOD.

By JOSEPH C. BOCK AND MAX GILBERT.

(From the Department of Physiological Chemistry, Marquette University, Milwaukee.)

Several drops of blood (80 to 150 mg.) are obtained from the tip of a finger or the lobe of the ear. The blood is collected in a small, thin glass tube and weighed. We prefer the use of a torsion balance, because the weighing can be done in such a short time that no anticoagulant is necessary.

The blood sample is transferred to a 4 ml. centrifuge tube and proteins are precipitated with a mixture of trichloroacetic acid and kaolin. After centrifugation and filtration an aliquot part of the filtrate is heated in a test-tube with a sulfuric acid digestion mixture. The ammonia is liberated by the addition of alkali, distilled by a micro steam distillation, and determined by Nesslerization.

THE DETERMINATION OF ARGININE BY THE USE OF ARGINASE, WITH APPLICATIONS TO THE ANALYSIS OF PROTEINS AND THE STUDY OF TRYPTIC DIGESTION.

By ANDREW HUNTER AND JAMES A. DAUPHINEE.

(From the Department of Biochemistry, University of Toronto, Toronto, Canada.)

Under suitable conditions arginase effects a practically complete conversion of arginine into ornithine and urea. It is therefore possible to determine arginine by the successive use of arginase and urease. A method based upon this principle has been applied (*a*) to the study of the rate at which arginine (or an arginine peptide susceptible to the action of arginase) is liberated during the course of tryptic digestion, and (*b*) to the determination of the arginine content of proteins.

(*a*) When 1 gm. of active "trypsin" is added to 250 cc. of a 5.5 per cent solution of *gelatin*, one-third of the total arginine is split off within the first 30 minutes; in 3 hours more than half has been liberated; and by the 3rd day an equilibrium has been reached, in which almost exactly two-thirds of the total arginine is free. A similar astonishingly rapid liberation of arginine has been observed in the digestion of *casein;* and here also just one-third of the arginine linkages appear to be resistant to the action of trypsin. With *edestin* the appearance of arginine is much more gradual; the process has not yet come to an end on the 6th day, at which point of time only 50 per cent is free.

(*b*) The arginase method may be applied to the solution of bases obtained in the Van Slyke method of protein analysis, where it possesses certain advantages over the usual procedure for the determination of arginine. As an example of the results obtained by its use, we have found for casein 7.7, 7.9, 8.0, 7.9, and 7.9 per cent of the total nitrogen in the form of arginine. The figure reported by Van Slyke (corrected, like our own, for the solubility of arginine phosphotungstate) is 8.1.

THE OCCURRENCE OF AMINO ACIDS AND OTHER ORGANIC NITROGEN COMPOUNDS IN LAKE WATERS.

By W. H. PETERSON, E. B. FRED, and B. P. DOMOGALLA.

(*From the Department of Agricultural Chemistry, University of Wisconsin, Madison.*)

Large quantities of water from Lake Michigan and four inland lakes of Wisconsin were centrifuged to remove plankton and bacteria, concentrated in a vacuum to 1/200 of the original volume, and analyzed for various forms of organic nitrogen.

Good color tests for proteins were obtained with ten different reagents. Proteins and their decomposition products were also shown to be present by precipitation with a number of protein precipitants. The existence of free amino nitrogen and peptide nitrogen was established by means of the Van Slyke, Folin, and Sörensen methods.

The quantity of certain amino acids was determined after hydrolysis of the concentrated sample and was found to range per cubic meter of water as follows: tryptophane from 5.5 mg. in Lake Michigan to 16.4 mg. in Green Lake; tyrosine from 8.3 mg. in Lake Michigan to 17.6 mg. in Devil's Lake; histidine from 5.7 mg. in Lake Mendota to 22.7 mg. in Turtle Lake; cystine from 1.5 mg. in Lake Mendota to 7.5 mg. in Turtle Lake.

Small quantities of amines and purines were also found. From 90 to 95 per cent of the organic nitrogen was accounted for by the different forms determined.

SOME OBSERVATIONS ON THE INTERRELATION BETWEEN THE FUNCTIONAL LEVELS OF THE ANIMAL BODY AND THE EXTERNAL COOLING POWER.

By E. S. SUNDSTROEM.

(*From the Department of Biochemistry, University of California, Berkeley.*)

Genetically related rats, which in every other respect were treated similarly, were given ample time to adapt themselves to a series of artificially produced climatic environments, which were maintained so as to give a gradient of cooling power; namely, (1) ordinary room temperature and humidity with air in motion (dry Kata 7, wet Kata 19), (2) high temperature and high humidity plus draft from electric fans (dry Kata 2, wet Kata 9), high temperature and low humidity (dry Kata 0.9, wet Kata 7), high temperature and low humidity, no "breeze" (dry Kata 0.8, wet Kata 4).

With the progressive drop in cooling power rather definite gradients were observed in the numerical data dealing with a number of morphological characters and functional levels. *Decreasing* proportionately with a lowered cooling power were: body weight, relative weight of internal organ, for instance liver, kidneys, spleen, and *thyroid*, resting respiratory metabolism, food consumption, blood sugar, blood uric acid, total creatinine of blood, nucleotide nitrogen of the blood, acid-soluble and lipoid phosphorus, and lecithin : cholesterol ratio. *Increasing* with the falling cooling power were: blood urea and to some extent amino acids, chlorides, partial blood cholesterol and relative weight of adrenals, body temperature of females (males preserved the body temperature better), and also a number of body indices, which appear to be positively correlated with the skin area. Besides, the coat of the white rats showed in the hot environments a reddish creamy coloration, the depth of which tended to increase along the cooling power gradient.

INORGANIC PHOSPHORUS IN INFANT BLOOD.

By ANTON R. ROSE, EDWIN A. RIESENFELD, and I. HANDLEMAN.

(*From the Prudential Life Insurance Company, Newark.*)

The arterial chord blood is consistently richer in inorganic phosphorus than venous chord blood. The average difference is only 4 per cent. The average of the inorganic phosphorus of the arterial and venous chord blood is in very close agreement with that of the mixed chord blood as usually taken (average difference 0.06 mg.). Blood samples from different anatomic levels will probably give different values. In a few cases the differences will be less than 10 mg. Between chord and fontanel the chances of one exceeding the other in inorganic phosphorus are about equal, but when the phosphorus of the fontanel is the larger in amount the differences are more pronounced, ranging from 10 to 75 per cent as against 10 to 30 per cent. The mass of blood cells runs parallel to the inorganic phosphorus so that if the percentage of blood cells be divided by milligrams of inorganic phosphorus per 100 cc. a constant number will be got which is nearly the same for all the samples of a given case, but not necessarily the same for different infants. The average of this constant for all infants comes to 13. The individuals range from 11.5 to 15.

A STUDY OF THE BIREFRINGENCE AND THE STAINING OF AGAR-AGAR AND OF GELATIN.

By JOHN FIELD, 2nd, and C. L. ALSBERG.

(*From the Food Research Institute and the Department of Chemistry, Stanford University, California.*)

The writers have shown recently that agar-agar dried in the gel form is very strongly birefringent, whereas dried in the sol form it is not birefringent at all except somewhat at the periphery or at the edges of a crack. It can now be reported that gelatin behaves in exactly the same way and that the phenomenon is not due to the production of strain by adhesion to the support during drying and shrinking. Preparations dried on oiled glass or on paraffin blocks do not adhere to them, yet show the properties above described. It is suggested that the birefringence of dried gels may need to be taken into consideration in interpreting some of the recorded observations upon the optical properties of dried gelatin.

The dried agar gels were quickly and deeply stained by alcoholic solutions of many of the dyes used in cytological studies, whereas the dried sols stained but slowly if at all. It is suggested that this phenomenon may be significant in cytological work. Furthermore, a hypothesis in explanation of the contrasting behavior of dried gels and sols, as recorded in the present investigation, is offered.

THE PURIFICATION OF JACK BEAN UREASE.

By J. B. SUMNER and V. A. GRAHAM.

(*From the Departments of Physiology and Biochemistry, Cornell University Medical College, Ithaca.*)

The authors have continued the work of Sumner, Graham, and Noback.[10] One part by weight of jack bean meal is extracted at 20°C. with 2 volumes of 30 per cent alcohol. The press-juice is made 35 per cent alcoholic and centrifuged. The centrifuged extract is allowed to stand in a tall cylinder overnight at −10°C. The supernatant liquid is syphoned off and discarded and the precipitate is centrifuged while cold. The precipitate, containing nearly all of the urease, is stirred up with 30 per cent alcoholic phosphate solution of the same pH as the original extract. The material is cooled at −10°C. for ½ hour and centrifuged. This process is repeated five times more and removes all carbohydrate and some protein. The precipitate is now stirred up with a small amount of dilute aqueous neutral phosphate solution and seeded with crystals of concanavalin A. After 48 hours at 3–5°C. all of the concanavalin A and concanavalin B will have crystallized out. These crystalline proteins are centrifuged off. The product has been dialyzed to remove phosphates, dehydrated with alcohol and ether, dried, and analyzed for total nitrogen and ash and for cystine, tyrosine, and tryptophane by the methods of Folin and Looney.[11] The results indicate that urease is of protein nature and that it is not identical with canavalin, concanavalin A, or concanavalin B. Urease can be purified further by conversion to insoluble urease by the action of dilute alcohol. The authors are at present engaged in analyzing insoluble urease.

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[10] Sumner, J. B., Graham, V. A., and Noback, C. V., *Proc. Soc. Exp. Biol. and Med.*, 1923–24, xxi, 551.

[11] Folin, O., and Looney, J. M., *J. Biol. Chem.*, 1922, li, 421.

PROTEINS OF THE BARK OF THE COMMON LOCUST TREE, ROBINIA PSEUDACACIA, LINN.

By D. BREESE JONES and C. E. F. GERSDORFF.

(*From the Protein Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.*)

The first recorded instance in which protein has been shown to occur in the bark of a tree is that noted by Power and Cambier,[12] who isolated from the bark of the common locust a protein having toxic properties. This protein was subsequently designated by the name *robin*. Numerous instances are recorded of both people and domestic animals having suffered as a result of chewing or eating the bark of this tree.

In view of the twofold interest connected with the protein of the locust bark, namely its toxic properties, and the fact that it opens a new and a wide field for protein investigation in a domain to which but little attention has been hitherto paid, we have undertaken a study of its proteins and other nitrogenous constituents.

The bark was stripped from the trees in August, and after carefully removing the outer corky portion, it was allowed to dry in the air, and finally ground to a coarse meal. The ground bark contained 2.8 per cent of nitrogen (moisture-free).

The results of exhaustive extractions of the bark made at room temperature are given in Table I.

TABLE I.

Percentages based on the moisture-free bark used.

Solvent.	Per cent of total N.	Proteins as per cent of the bark (N × 6.25).
Distilled H_2O	54.69	9.57
10 per cent aqueous NaCl	9.36	1.64
70 " " alcohol	0.67	0.12
0.5 " " aqueous NaOH	1.34	0.23
Total	66.06	11.56

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[12] Power, F. B., and Cambier, J., *Pharm. Rundschau*, 1890, viii, 29.

There have been isolated from the bark 2.52 per cent of albumin, 1.38 per cent of a globulin, and a quantity of a substance having the properties of a proteose.

The albumin is completely precipitated from a 10 per cent salt solution by making the solution 0.4 to 0.5 saturated with ammonium sulfate. On heating its salt solution the albumin coagulates at 61° to 62°C. The average results of elementary analyses of nine different preparations showed the albumin to have the following composition: C 54.52, H 6.83, N 14.76, S 0.80.

The distribution of nitrogen and percentages of diamino acids in the albumin as calculated from results obtained by the Van Slyke method of analysis are expressed in Table II as percentages of the protein. Cystine, tryptophane, and tyrosine were determined colorimetrically.

TABLE II.

Locust Bark Albumin.

	per cent
Amide N	1.41
Humin N	0.53
Basic N	3.09
Non-basic N	9.95
Cystine	1.37
"	1.03*
Arginine	4.39
Histidine	1.74
Lysine	5.45
Tryptophane	4.18†
Tyrosine	6.27*

* Determined by the colorimetric method of Folin and Looney (Folin, O., and Looney, J. M., *J. Biol. Chem.*, 1922, li, 421).

† Determined by the colorimetric method of May and Rose (May, C. E., and Rose, E. R., *J. Biol. Chem.*, 1922, liv, 213) with certain modifications.

Further investigation of the proteins of the locust bark is in progress.

THE FATTY ACIDS OF BLOOD PLASMA.

By W. R. BLOOR.

(*From the Department of Biochemistry and Pharmacology, The University of Rochester, Rochester, New York.*)

According to our present belief, the unsaturated fatty acids in the animal body represent stages in the oxidation of the fatty acids of the fats of the food and stores. Consequently their occurrence, mode of combination, and relative proportions in the various tissues and organs become a matter of importance in the consideration of fat metabolism. In earlier work, data on their occurrence and distribution in the blood plasma have been reported, and the present communication is a report on attempts to determine their nature and relative proportions. The work indicated that of the fatty acids in all types of combination in blood plasma, oleic and linolic in varying proportions constitute the main constituents with palmitic acid next, then a small amount of a four double bond acid, and considerably less of an acid with three double bonds.

THE INFLUENCE OF DIET ON FAT PRODUCTION IN THE ANIMAL BODY.

By WILLIAM E. ANDERSON.*

(From the Laboratory of Physiological Chemistry, Yale University, New Haven.)

We have approached the problem of fat production in the animal body by means of the more recent methods of animal feeding, by which due consideration can be given to each of the following factors: (1) total calorific value of ration; (2) variations in the protein with respect to its biological "quality," and the plane of concentration in the ration at which it is fed (this involves the so called nutritive ratio); (3) content and character of the carbohydrates fed; (4) fat factors furnished preformed in the diet; (5) the mineral nutrients with particular reference to deficiency of any individual element; and (6) the indispensable vitamins.

In order to determine the nature of the fat deposited under different conditions of "controlled" diet and to determine something regarding its chemical character, the fat is rendered from the entire eviscerated animal.

Peanut oil, cottonseed oil, soy bean oil, corn oil, cocoanut oil, Crisco, lard, and butter fat, which represent oils and fats of a widely varied character, have been fed in different diets, and the quality of the resulting body fat has been examined in rats ranging in weight from 140 to 250 gm. The results obtained thus far have clearly shown the specific influence of the different oils and fats fed, as judged by the variations in the iodine number and refractive index values.

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* Fellow of the Institute of American Meat Packers.

By way of contrast the effect of a diet rich in carbohydrate and containing less than 1 per cent of fat has been studied. Both young and mature animals raised on this diet yield a comparatively hard fat. Furthermore, when the diet of rats raised to 135 and 150 gm. is changed from one containing peanut oil, cottonseed oil, soy bean oil, and corn oil to this high starch diet, mature rats produce fats which compare very closely in texture with the fats obtained from rats fed on the high starch diet. When a diet high in protein is fed, the fat produced is very similar to that laid down on the high carbohydrate diet.

THE FATTY ACIDS EXCRETED BY DOGS ON A FAT-FREE DIET.

By W. M. SPERRY.

(From the Department of Biochemistry and Pharmacology, The University of Rochester, Rochester, New York.)

In connection with a study of the lipoids excreted by dogs on a fat-free diet during a period of 5 weeks, the fatty acids were separated and their composition determined. Despite the fact that the composition of the volatile acids seemed fairly constant, averaging about 65 per cent acetic, 20 per cent butyric, and 15 per cent caproic when analyzed by the Duclaux method as modified by Gillespie and Walters, no direct relationship could be shown between the amounts of the volatile and non-volatile fractions. Moreover, no connection was observed between the amounts of the non-volatile fractions and the weights of the animals. Although the dogs varied between 5.3 and 12.9 kilos in weight, the non-volatile fatty acid fraction averaged 1.012 gm. per dog per week with a maximum variation of 0.641 gm. and an average variation of 0.216 gm. The liquid acids were always excreted in greater amount than the solid, there being in most cases 1½ to 2 times as much of this fraction present. Separation and analysis of the bromides indicated that the liquid acids consisted almost entirely of an acid with one double bond with small amounts of an acid with three double bonds always present.

THE FORMATION OF LACTIC ACID BY THE DIABETIC ORGANISM.

By EDWARD A. DOISY, A. P. BRIGGS, C. H. WEBER, AND IRENE KOECHIG.

(From the Laboratories of Biological Chemistry of St. Louis and of Washington Universities, St. Louis.)

In agreement with the results of Barr and his collaborators on phlorhizinized dogs, we find that depancreatized dogs form large amounts of lactic acid during muscular exercise. Our data indicate that as long as the muscles contain glycogen, lactic acid is formed during contractions.

To supplement these data we have studied the chemical changes in the stimulated muscles of phlorhizinized frogs. Preliminary results on the mechanism of contraction indicate a normal utilization of carbohydrate or lactic acid in the oxidative recovery. The percentage of total fatty acids was not altered.

TYPES OF TOLERANCE FOR DIFFERENT CARBOHYDRATES (GALACTOSE, PENTOSES).

By HILDING BERGLUND AND TSANG G. NI.

(From the Department of Biological Chemistry, Harvard Medical School, Boston.)

The normal tolerance for galactose after oral administration to man shows great individual variations unknown for glucose or fructose. The degree of galactose tolerance in a given individual is constant when tested on different occasions. Above the limit of tolerance, rapidly increasing loss of galactose occurs with increasing amount taken.

The behavior of pentoses is entirely different from that of the hexoses. The percentage lost in the urine seems to be essentially constant, independent of the amount injected.

THE EFFECT OF FASTING UPON CARBOHYDRATE UTILIZATION.

By ELMER L. SEVRINGHAUS.

(From the Department of Physiological Chemistry, University of Wisconsin, Madison.)

In the first 2 hours after an ordinary meal there is first a moderate hyperglycemia, then a return to normal blood sugar concentration. In many individuals there is immediately following this a hypoglycemia of varying degree and duration. Usually there is a final return to the "fasting" level in about 2 hours after the meal. Some persons show a delayed relief from hypoglycemia, and a corresponding marked hunger. Hunger is not correlated with any definite blood sugar level, or necessarily with a subnormal blood sugar concentration. If the same meal is given three times daily, the reaction to each meal is similar. No difference is observed when a fast of 24 to 36 hours intervenes between meals. But when the fasting is for 2 days or longer the blood sugar curve after the meal is entirely different. The hyperglycemia is more pronounced, and there is little tendency to return to normal blood sugar level in the first 2 hours. The eventual return is slow. The curve suggests the "sugar tolerance curve" of the very mild diabetic. It is interpreted as indicating delayed pancreatic response. Ketosis is found to be present when this abnormal response to the meal occurs. The shorter fast induces no ketosis. Ketosis or possibly some other product of fasting seems to inhibit either the production or the action of insulin.

THE THRESHOLD OF KETOGENESIS IN PREGNANCY.

By VICTOR JOHN HARDING, KATHLEEN DREW ALLIN, ALFRED BLYTHE EAGLES, and H. B. VAN WYCK.

(From the Department of Pathological Chemistry, University of Toronto, and the Metabolic Ward of the Burnside Wing, Toronto General Hospital, Toronto, Canada.)

Diets in which 74 to 78 per cent of the total calories were obtained from fat were fed to women in various stages of pregnancy. Such diets are at the threshold of ketonic production when fed to non-pregnant individuals, and controls with non-gravid women showed only small amounts of "acetone" excretion per day over a period of 8 days. Thirteen experiments on pregnant women showed excretions of acetone varying from 2 to 12 gm. of "total acetone" a day.

The composition of the burning metabolic mixture was calculated from the total calories (basal metabolism + 20 per cent increase for movements in bed and the specific dynamic action of protein), from the urinary N, and assuming the complete combustion of the carbohydrate. The ketogenic-antiketogenic ratios calculated according to Shaffer[13] showed ratios varying from 1:0.45 to 1:0.87. Under these circumstances no acetone should be formed. Reckoned according to Shaffer's earlier calculations[14] the ratios lie between 1:0.86 and 1:1.38. Compared with normal non-pregnant individuals[15] when on diets of similar composition the threshold of ketogenesis in pregnancy appears to be low. This is taken to be true as an average result. Until further work has been carried out on non-gravid women as controls, and under conditions strictly comparable with the experiments in pregnancy, the authors, however, hesitate to regard it as true in all cases. The factors which can influence the production of ketonuria are so many that strictly accurate comparison of the two states is only possible with a larger number of cases than those as yet studied.

THE EFFECT OF INSULIN ON THE RESPIRATORY METABOLISM.

By WALTER M. BOOTHBY AND ROBERT WEISS.*

(From the Section of Clinical Metabolism, Mayo Clinic and Mayo Foundation, Rochester, Minnesota.)

We have carried out approximately 50 experiments on men and dogs to study the effect of insulin on the respiratory metabolism. We have confirmed the original observations of Boothby and Wilder that insulin does not increase the rate of heat production in man if given in amounts which do not decrease the blood sugar to the point where a hypoglycemic reaction is produced. On the other hand, if a reaction develops, an increase in metabolism occurs even when no movements are made and no demonstrable change in tonus occurs. The most satisfactory subject upon which to show this phenomenon is a diabetic patient with a high blood sugar. After 30 units of insulin the blood sugar of one of Dr. Wilder's patients dropped from 190 to 70 mg. per 100 cc. during 140 minutes and the heat production remained constant until the sudden development of the typical hypoglycemic reaction at 69 mg. when the fall in blood sugar suddenly stopped;

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[13] Shaffer, P. A., *J. Biol. Chem.*, 1922, liv, 399.

[14] Shaffer, P. A., *J. Biol. Chem.*, 1921, xlvii, 449.

[15] Hubbard, R. S., and Wright, F. R., *J. Biol. Chem.*, 1922, l, 361.

* Fellow in Medicine of The Rockefeller Foundation.

with the reaction the metabolism increased. In dogs we invariably obtained an increase in heat production of 10 to 20 per cent coincident with a fall in blood sugar which would probably cause a hypoglycemic reaction. It was anticipated that the introduction of glucose through a gastrostomy wound at a rate sufficient to keep the blood sugar from dropping, would prevent the increase in heat production; such, however, was not the case in a series of six experiments although the control experiment with the same quantity of glucose alone without the insulin caused no increase.

In one dog during the course of these insulin experiments, we have been able to elevate the basal respiratory quotient by high carbohydrate diet to 1.10. Regardless of the initial level of the basal respiratory quotient the administration of insulin still further raises the quotient. It is impossible in dogs to run the blood sugar and CO_2-combining power at the same time as the metabolism; in control experiments, however, we get in general, but not invariably, a drop of 4 to 7 volumes per cent in the CO_2-combining power after 12 units of insulin. It can be calculated from our data for the dog that the gradual decrease during 90 minutes of 7 volumes per cent will increase the CO_2 elimination per minute by 4 or 5 cc. which with an oxygen consumption of 70 cc. per minute will elevate the quotient approximately 0.07; for example, from 0.90 to 0.97. Likewise on man, we frequently obtain a drop of 5 volumes per cent in CO_2-combining power after insulin. It is obvious that until further quantitative relationships are established between the CO_2-combining power and the rise of the respiratory quotient it is not justifiable to draw deductions from the respiratory quotient in regard to the effect of insulin on the metabolic processes.

THE INFLUENCE OF INSULIN, ADMINISTERED ORALLY AND SUBCUTANEOUSLY, IN PHLORHIZIN DIABETES.

By O. H. GAEBLER.

(From the Department of Vital Economics, The University of Rochester, Rochester, New York.)

The influence of insulin introduced subcutaneously and orally into phlorhizinized dogs has been studied in twelve subjects. Phlorhizin was administered by the Coolen method (in olive oil) once or twice daily. All urine periods were separated by catheter. Sugar was determined by Benedict's method, and nitrogen by the Kjeldahl-Gunning method. Blood sugars were taken only occasionally and were determined by the Folin and Wu or by the Hagedorn-Jensen method. The respiratory metabolism was followed in seven subjects by means of a closed circuit apparatus, including a chamber of the Benedict type. The insulin was made in this laboratory by the rapid method, *i.e.* amyl alcohol precipitation, and for oral administration was made into enteric coated tablets or pills from already mixed material supplied by the Sharp and Dohme laboratories in Baltimore, or was combined as desired in the laboratory with various substances which might be supposed to delay the destructive action of trypsin. Two animals only of the twelve were fed on a regular diet of lean beef heart. Dogs 6 to 11 inclusive were given glucose (Merck's C.P. dextrose) in exact amounts every day by stomach tube. The effects of insulin on the hourly excretion, as well as total for 24 hours, of dextrose and nitrogen were studied simultaneously with the respiratory quotient.

CONCLUSIONS.

1. Insulin introduced subcutaneously into dogs completely under the influence of phlorhizin acts immediately to cause a reduced rate of excretion of sugar, and an increase in the respiratory quotient.
2. When glucose is given the rate of excretion of nitrogen is reduced (protein is spared), but when glucose and insulin both are given it is reduced still more. These two fractions represent the "dextrose" and "complementary nitrogen" of A. I. Ringer.
3. Insulin administered orally in enteric coated tablets, combined with malic acid, sodium oleate, and an amino acid which liberates HCl, has given positive results on the reduction of urine sugar, increase in the R. Q., and sparing of protein. The effect is much less in amount than from subcutaneous administration.
4. The sugar which disappears from the urine and does not reappear may amount to from 0.28 to 0.48 gm. per clinical unit (1/3 of "Rochester Unit") of insulin administered subcutaneously.

No exact estimate could be made after oral administration, but, of course, it is very much less than after subcutaneous use.

5. Some of the sugar which disappears from the urine may reappear when the insulin effect wears off, because, as Cori has shown for the rabbit, it may be stored temporarily as glycogen.

THE INFLUENCE OF INSULIN AND EPINEPHRINE ON THE LACTIC ACID CONTENT OF BLOOD AND TISSUES.

By CARL F. CORI.

(From the State Institute for the Study of Malignant Disease, Buffalo.)

In view of the importance of lactic acid as an intermediary product of carbohydrate metabolism an investigation of the influence of insulin and epinephrine on the lactic acid content of blood and tissues was undertaken. The experiments were carried out on rabbits, cats, on one dog, and on mice. The mice were used for the determination of the lactic acid in the tissues, while the changes in the lactic acid of the blood were followed in the other animals.

The normal lactic acid content for rabbits (twelve experiments) varied from 13.2 to 39.7 mg. per 100 cc. of blood. Insulin given to fasting rabbits, to fasting and phlorhizin-poisoned rabbits, and insulin followed by glucose in order to relieve hypoglycemic symptoms, did not produce a significant change in the lactic acid content of the blood. However, a few minutes after a strong convulsive seizure high lactic acid values up to 106 mg. per 100 cc. of blood were found. Epinephrine caused in rabbits a decided rise in the lactic acid content of the blood. The normal lactic acid values for cats (fifteen experiments) varied from 15.1 to 34.1 mg. per 100 cc. of blood. Insulin was given to fasting normal cats, to fasting depancreatized cats, or insulin was followed by glucose in order to relieve hypoglycemia, but no decided change in the lactic acid content of the blood was recorded. Epinephrine caused an increase in the lactic acid content of the blood, but the response was not as constant nor as marked as in rabbits.

The lactic acid content of the liver and the muscles of mice was determined during moderate insulin hypoglycemia, during insulin convulsions or coma, and during epinephrine hypoglycemia. A control mouse was always killed as simultaneously as possible with an injected mouse. There was no decided difference between the lactic acid content of the tissues of the control animals and of the injected animals. Glycogen and lactic acid maximum determinations showed that even during insulin convulsions there were enough reserve carbohydrates present in the liver and the muscles to give much higher lactic acid values than were actually found.

AN INTERPRETATION OF THE BIOLOGICAL REDUCTION OF METHYLENE BLUE.

By W. MANSFIELD CLARK, BARNETT COHEN, AND H. D. GIBBS.

(*From the Division of Chemistry, Hygienic Laboratory, United States Public Health Service, Washington.*)

The view is advanced that the reductive process of the cell is a unique characteristic of life for the study of which there still have to be developed adequate concepts, reliable methods of investigation, and sets of exact quantitative data.

This contribution is the establishment of equilibrium values for the reversible reduction of the most widely used indicator, methylene blue.

It is also established that the two electrochemical equivalents concerned in the oxidation-reduction process are associated with exactly the same energy intensity, and that the hydrogens entering into the ordinary formalistic equation of reduction are associated with ionization constants of enormous differences. This is inconsistent with Wieland's theory.

While no conclusive proof of mechanism is established it may be inferred that the reduction of methylene blue consists of the transfer to this oxidant of an electron pair. This may or may not be followed by one or the other components of water according to the acid-base equilibrium state of the solution.

Electrode potential measurements with cell suspensions (bacteria and liver) give data agreeing with those calculated from the data for the reduction of methylene blue.

The potentials thus established by two methods lie in a zone where there can be no appreciable quantity of either molecular hydrogen or molecular oxygen in *equilibrium* with the system.

If either gas be present it can be considered only in relation to a dynamic process and not to a static equilibrium.

A STUDY OF ALL-DAY BLOOD SUGAR CURVES IN NON-DIABETIC INDIVIDUALS AND IN DIABETIC PATIENTS TREATED WITH AND WITHOUT INSULIN.

By LEON JONAS, T. GRIER MILLER, and IDA TELLER.

(*From the William Pepper Clinical Laboratory, University of Pennsylvania, Philadelphia.*)

As a result of the study of thirty-four curves plotted to show the concentration of the blood sugar at frequent intervals over varying periods of time in both non-diabetic and diabetic individuals on various diets it is concluded: (1) that the dosage of insulin and the time of its administration to patients with diabetes must be controlled by a consideration of the concentration of the blood sugar throughout the entire day; (2) that a curve showing roughly this full day concentration can be constructed in most cases by having the patient on a diet of equal value as to the protein, fat, and carbohydrate content at each of his three regular meals and by determining the blood sugar level before breakfast, or before the morning administration of insulin if that is being employed, an hour after breakfast, and just before luncheon; and (3) that the ideal method for the management of such cases has not yet been arrived at, but that the following general rules for the administration of insulin to those patients with diabetes who require it seem warranted.

With a maintenance diet equally distributed among the three meals of the day mild cases may be kept within the normal limits of glycemia by means of a single dose administered a half hour before breakfast.

More severe cases require in addition to an adequate morning dose a second but somewhat smaller one a half hour before the evening meal.

When the morning fasting level of blood sugar cannot be kept below the threshold value for glycosuria by these two doses a third one is indicated at about midnight.

OBSERVATIONS ON BLOOD PHOSPHATES AS RELATED TO CARBOHYDRATE METABOLISM.

By A. BOLLIGER and F. W. HARTMAN.

(*From the Laboratories of the Henry Ford Hospital, Detroit.*)

The inorganic phosphates of the blood are depressed during carbohydrate metabolism if insulin is available. This is best shown in the comparative studies on normal, partially depancreatized, and completely depancreatized dogs.

The earliest defect in pancreatic function is demonstrated in a retarded but prolonged depression of the curve of inorganic blood phosphates. The complete absence of functioning pancreas is shown by the blood phosphate level which remains unaffected as a straight line during carbohydrate metabolism.

Carbohydrates and phosphates are best utilized when there is an excess of each in the circulation.

Medullary puncture and epinephrine effect a hyperglycemia with a corresponding fall in the inorganic blood phosphates.

As opposed to epinephrine, pituitrin produces an immediate and sharp increase of the blood phosphates along with the usual hyperglycemia. This gives additional support to the view that stores of carbohydrates and phosphates are actively broken down.

THE DISTRIBUTION OF THE UNSATURATED FATTY ACIDS, CHOLESTEROL, AND CHOLESTEROL ESTERS IN EXPERIMENTAL ANEMIA.

By MEYER BODANSKY.

(*From the Laboratory of Biological Chemistry, University of Texas, Galveston.*)

In acute experimental anemia due to acetylphenylhydrazine or symmetrical diisopropylhydrazine, the total cholesterol in the plasma is not markedly affected, while in the corpuscles it is usually increased. Cholesterol esters are absent from the corpuscles of normal dogs, but appear in relatively high concentration in the corpuscles of anemic dogs. Of the total cholesterol in the corpuscles, as much as 45 per cent has been found in the form of esters. Cholesterol esters in the corpuscles have been observed, likewise, in the mild anemia which follows splenectomy.

The concentration of the unsaturated fatty acids in the plasma and whole blood remains within physiological limits in experimental anemia, but in the corpuscles, the concentration may be as much as doubled. The altered cóntent of the unsaturated fatty acids was most marked when the anemia was associated with an increased volume and decreased hemoglobin concentration of the individual corpuscles. The presence of cholesterol esters and of increased amounts of unsaturated fatty acids indicates that the individual corpuscle in anemia may be concerned with a proportionately greater amount of lipoid transformation than normally.

It does not appear that the unsaturated fatty acids play any significant rôle in blood destruction. This is made evident especially in symmetrical diisopropylhydrazine anemia. The initial effects are severe fatty degeneration of the liver accompanied by anhydremia, the anemia developing subsequently. Even during the height of the intoxication, no significant variations from the normal concentrations were observed in the case of the unsaturated fatty acids of the blood.

CUMULATIVE TESTS OF THE POSSIBLE TOXICITY OF INTARVIN, WITH NOTES ON ITS UTILITY IN THE TREATMENT OF DIABETES.

By HATTIE L. HEFT, MAX KAHN, and WILLIAM J. GIES.

(From the Laboratories of Physiological Chemistry at Teachers College, and of Biological Chemistry at the Beth Israel Hospital, and the School of Medicine of Columbia University, New York.)

Two series of experiments on albino rats, through successive generations, have been conducted to determine whether intarvin, added to a *natural* balanced diet, in quantities ranging from 5 to 12 per cent of the total daily food intake, manifests toxicity. Eight normal female rats from one litter were originally separated into groups of four, and one of two males of practically the same size and vigor from an unrelated litter was added to each group. One such group received the balanced diet with a definite proportion of intarvin; the other received the balanced diet, with rendered lamb fat instead of intarvin added to it. In each successive generation, thereafter, the maternal rats that received intarvin were selected from the direct descendants of those that had been receiving the "intarvinated" diet. The control maternal rats were direct descendants of those that received a diet containing an addition of lamb fat. The paternal rats were not related to the prospective mothers, but were selected on the same dietary basis as that for the maternal, or from litters on the balanced diet without addition of fat.

Rats have already been carried, in this parallel manner, into the seventh generation. There have been no discernible effects on the animals thus treated, nor on their fecundity, at any stage of the tests. Similar experiments, with intarvin the only fatty ingredient of synthetic diets, are under way.

Further study of the beneficial effects of intarvin in the treatment of diabetes has fully confirmed the earlier indications of its utility and its lack of toxicity, even when eaten to excess. It has been fed to patients daily for more than 2 years, and has induced neither gastrointestinal derangement nor other indications of toxicity in any of them. It is absorbed to the extent of 95 per cent of the amount eaten; it is not only non-ketogenic but sometimes acts as an antiketogenic agent; and its metabolic products never increase the general acidosis of the organism.

After eating intarvin continuously, in adequate amounts, diabetic patients gain in weight and strength, and lose their sense of hunger. Intarvin complements the action of insulin, and often reduces the dosage of insulin. Intarvin may be used advantageously in diabetes, whether the patient receives insulin or not. The dietetic minimum of intarvin in diabetes is the quantity that will meet the caloric maintenance requirement. Further clinical study is actively in progress.

THE CHEMICAL AND BIOLOGICAL PROPERTIES OF PURE ARSPHENAMINES.

By PHILIP ADOLPH KOBER.

(*Nepera Park, New York.*)

The elimination of methyl alcohol in arsphenamine, using the hydrochloric acid method described by the author in 1919, produces a uniform product of high purity, containing 0.3 per cent of sulfur, a trypanocidal power of about 8.5 mg. per kilo, with a toleration of about 190 mg. per kilo, light yellow in color, non-hygroscopic, with strong gel-forming tendency, easily soluble in warm water, and insoluble in methyl alcohol. Most arsphenamine and salvarsan preparations of to-day are of the type easily soluble in cold water and in methyl alcohol, distinctly different from Ehrlich's first preparation and from pure arsphenamine. Laboratory evidence indicates that these easily soluble types may be as much as 50 per cent less potent than a pure product. Chemical evidence supports the theory that this change of character is due to the methyl alcohol in the final product having combined with the base as a methyl derivative of some sort. Reprecipitation of these methyl alcohol arsphenamines with hydrochloric acid raises in most preparations their potency and lowers their toxicity, and also their sulfur content.

Pure neoarsphenamine, having 30 to 31.5 per cent of arsenic depending on the amount of water of crystallization left in it, yellow to orange color depending on the size of crystal, is very much like pure arsphenamine: (1) slowly but not difficultly soluble in water, with some gel formation; (2) a like trypanocidal power of about 9.0 mg. per kilo; and (3) toleration in rats approximately equal to that of arsphenamine, 185 mg. per kilo. Neoarsphenamines in this country as in England in 1922 were made so as to be administrable in concentrated solutions without clinical reactions; *i.e.*, very mild. In common with the original neosalvarsan these neoarsphenamines have only 18 to 20 per cent of arsenic and contain approximately 40 per cent of impurities. These impurities do not, as does methyl alcohol in arsphenamine, increase the toxicity, but on the contrary, decrease it. This decrease in toxicity may be as much as 300 per cent. The result of making the neoarsphenamines innocuous is likewise to diminish the curative power, as much as 300 per cent and more. In other words, the character of the drug has been changed.

These mild arsphenamines, made not for their purity, but because they are, particularly the neoarsphenamines, the most innocuous, are such deviations from the curative essence or base, that it is a fair question whether or not the Public Health Service, whose regulations pay no attention to chemical purity or potency, should not call a halt to the manufacture and sale of such semi-fraudulent preparations, or insist on their being accurately labelled or whether the trustees of the basic patents would not be justified in withdrawing the license from those whose use of the patents results in products so different than the products intended by the inventor and so contrary to public health interests.

All chemical and biological evidence proves that arsphenamines themselves, and not their impurities only, have a certain toxic power as well as a certain curative effect, and any attempts to deny this or to alter these properties in these drugs, are as reasonable as to attempt to take the poisonous qualities out of strychnine or any other powerful drug. If milder derivatives are found to be useful, let them be called and treated as new products, which in truth they are.

THE RELATION BETWEEN THE UNDETERMINED NITROGEN OF THE BLOOD AND ITS TOXICITY TO LUPINUS ALBUS SEEDLINGS.

BY JOSEPH M. LOONEY AND DAVID I. MACHT.

(From the Biochemical Laboratory of the Sheppard and Enoch Pratt Hospital, Towson, and the Pharmacological Laboratory of Johns Hopkins Medical School, Baltimore.)

In a recent communication by one of us[16] it was shown that the undetermined nitrogen was increased in the blood of markedly depressed patients. This was taken as an indication of the presence of toxic amines.

While this work was in progress there was published by the other author[17] a paper in which it was demonstrated that there was present in menstrual serum a toxin that decreased the growth of *Lupinus albus* seedlings in Shive solution from 75 to 51 per cent.

It was thought that the blood of these psychotic patients might be tested for the presence of toxic products in a similar manner. We have estimated the undetermined nitrogen and the coefficient of growth of the *Lupinus albus* seedlings in the blood of a number of patients.

The averages of these figures for nineteen toxic and eight non-toxic cases are given in Table I.

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[16] Looney, J. M., *Am. J. Psychiat.*, 1924, iv, 29.

[17] Macht, D. I., and Lubin, D. S., *J. Pharmacol. and Exp. Therap.*, 1922, xxii, 413.

TABLE I.

Blood from Psychotic Patients.

	8 cases non-toxic to *Lupinus albus.*	19 cases toxic to *Lupinus albus.*
Undetermined nitrogen (*a*), *per cent non-protein N*	14.0	19.5
Index of growth (*b*), *per cent*	75.0	53.5
Factor ($a \times b$)	1,050	1,040

The third line gives the factor resulting from multiplying the percentage of undetermined nitrogen by the percentage of growth for both groups. The constancy of this factor is quite striking and offers very remarkable support to the idea that the toxicity of the bloods varies with the undetermined nitrogen.

It is to be noted that this toxin is evidently very different from that found in menstrual serum which is possibly due to a derivative of cholic acid,[18] as the specimens taken were from women who were not menstruating or who had passed the menopause and from men; some of the most toxic specimens being from men.

A STUDY OF THE ENERGY AND SUBSTANCE METABOLISM OF UNDERNOURISHED CHILDREN.*

By CHI CHE WANG, RUTH KERN, MARGARET FRANK, and JEANNETTE DUNWIDDIE.

(*From the Nelson Morris Memorial Institute for Medical Research, Michael Reese Hospital, Chicago.*)

Thirty-one undernourished children, ranging in age from 4 to 13 years, served as subjects under well controlled conditions. They were divided into three groups based on percentage underweight according to their height, varying from +6 to −26 per cent. Four of them were slightly above normal height, two exactly normal, and the other twenty-five were under height, ranging from 1 to 15 cm. below normal. All the children looked pale and sickly.

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[18] Macht, D. I., and Hyndman, O. R., *J. Pharmacol. and Exp. Therap.*, 1924, xxii, 483.

* Aided by a fund contributed jointly by the Elizabeth McCormick Memorial Foundation, Mrs. Francis Nielson, and Mrs. Gusta Morris Rothschild.

Basal metabolism was determined by means of the Benedict chamber bed and Universal respiration apparatus. There is no difference in the basal metabolism of the three groups, computed on the basis of total calories or surface area referred to either age or weight, but in all these cases the figures were higher than the Benedict standards. An interesting observation was that the basal metabolism of these children was comparable with that predicted for children of their age who were normal in height and weight.

The energy value of excretions was determined by means of Benedict's bomb calorimeter. The total loss varied from 108 to 199 calories per 24 hours, but here again there is no difference in the three groups. On the whole, the most underweight children had a higher per kilo intake and hence a higher caloric utilization per kilo of body weight. The calorie: nitrogen ratio in all groups ranged between 7.1 and 12.2, which is within normal range according to Utheim.

The per kilo nitrogen intake, in agreement with the calorie intake, shows very little difference in the three groups. The average of each group is, however, higher in the more undernourished children, being 0.431, 0.465, and 0.514 gm., respectively. The average value for absorption per kilo of body weight follows in the same manner, namely 0.386, 0.413, and 0.466, but the nitrogen retention is only 0.069 gm. in the third group as compared with 0.087 and 0.089 gm. in the other groups. This tends to indicate that food is not so well digested by the more undernourished children, but more is excreted through the feces.

Calcium metabolism shows no difference among the three groups, in intake, output, utilization, or retention.

All the children showed a creatinuria, that of the first and second groups averaging 7.5 and 7.6 mg. per kilo, and that of the third group 9.2 mg. per kilo. On the other hand, creatinine per kilo is practically constant in the three groups, averaging 20.6, 21.9, and 21.7 mg. per kilo, respectively. The creatinine coefficient is 7.9 for the three groups, which is comparable with reported figures for adults. Ammonia nitrogen was well within normal limits in all cases, as was also the ammonia nitrogen: total nitrogen ratio.

The lack of variation from normal may be due to the fact that these children are not sufficiently undernourished to be really emaciated. Only three cases are more than 20 per cent underweight. The likelihood is that they are deficient simply in their fat stores, and that their actual tissue metabolism still approximates that of normal children.

THE INFLUENCE OF INFECTION ON THE LIPOIDS OF THE SUPRARENAL GLAND.

By EMIL J. BAUMANN and OLIVE M. HOLLY.

(From the Division of Laboratories, Montefiore Hospital, New York.)

Normal suprarenals were compared, in regard to cholesterol, phosphatide, and fat, with the suprarenals taken from animals that died, or were killed, because of infections.

In the suprarenals of normal guinea pigs the average "fat" (total ether-alcohol-soluble matter less cholesterol and phosphatides), cholesterol, and phosphatide content was 7.7, 3.7, and 1.8 per cent, respectively, while in animals that died of various infections the corresponding average values were 11.2, 1.0, and 2.2 per cent. The most characteristic and uniform difference is the marked loss of cholesterol.

Similar observations were made on rabbits. Unlike the uniform figures found for guinea-pigs, the variations among normal animals were very large. This can be explained in part by the fact that the stock had been exposed to snuffles, although in the animals used for "normals" no nasal mucous discharge had been noted during the period of observation. In spite of this very serious complicating factor several deductions may be made from these data.

The average "fat," cholesterol, and phosphatide percentages of the suprarenals of twenty-six normal rabbits were, respectively, 13.2, 8.8, and 2.5 per cent. The corresponding values in the case of nine rabbits that died of pneumonia, peritonitis, etc., were 13.8, 4.8, and 2.8 per cent. Thirteen rabbits, sacrificed because of obvious illnesses, which proved on necropsy to be similar infections, show averages of 16.0, 11.1, and 2.2 per cent, respectively.

It would appear that during the early stages of these infections an *increase* in the cholesterol content of the suprarenal occurs, which is followed by a decrease later in the disease. The fact that a rise of the percentage of cholesterol occurs is more strikingly brought out by the figures obtained from analyses of the suprarenals of four rabbits that were sacrificed because of middle ear infections—comparatively a very mild and localized infection. The average figures for "fat," cholesterol, and phosphatide were 8.5, 20.6, and 2.6 per cent, respectively.

ON SPECIFIC SPERM AGGLUTININS.

By G. H. A. CLOWES and EDA B. WALDEN.

(From the Biochemical Research Laboratory, Eli Lilly and Company, Indianapolis.)

The substance derived from sea urchin eggs by aqueous extraction, which is capable of exerting a specific agglutinating action on sea urchin sperm, may be profoundly modified as regards its susceptibility to heat and various chemical reagents by extracting certain lipoids from the solutions containing the agglutinin or by treating the eggs in advance with appropriate concentrations of certain organic reagents.

These results harmonize with those previously observed by the writer regarding the influence exerted by lipoids on the heat sensitivity of pollen toxins, and appear to throw some light on the physical constitution of protoplasmic particles.

A COLORIMETRIC METHOD OF DETERMINATION OF BILE SALTS IN THE BLOOD.

By SHIRO TASHIRO.

(From the Biochemical Department, University of Cincinnati, Cincinnati.)

Pettenkoffer's method of detection of bile salts is so regulated that the color developed is permanent enough to give ample time for colorimetric estimation. With a proper process of extraction, this method is sensitive to measure bile salts in blood in a dilution as small as 5 in 100,000. A simple method of preparing permanent standards, which give a perfect match to the color developed with Pettenkoffer's method with the bile salts, will be given.

THE COLORIMETRIC DETERMINATION OF TRYPTOPHANE BY THE VANILLIN-HCl REACTION AND THE QUANTITATIVE SEPARATION FROM INDOLE AND SKATOLE.

By IDA KRAUS.

(From the Hull Laboratories of Physiological Chemistry, University of Chicago, Chicago.)

The color reaction here employed distinguishes indole and skatole from tryptophane by the differences in the colors obtained. Indole and skatole can be removed quantitatively by extraction with toluene before the reaction is applied. Tryptophane can thus be recovered quantitatively. Hydrolysis of a protein by barium hydroxide not only destroys tryptophane, but also brings about other decompositions which alter the aldehyde condensation reactions. Barium hydroxide hydrolysis of proteins and of tryptophane respectively produces different decomposition products which are reactive with tryptophane reagents. These products are precipitated by mercuric sulfate. The barium hydroxide hydrolysis of pure tryptophane actually causes a loss of 7 to 20 per cent thereof, depending on whether the phenol reagent of Folin and Looney or the vanillin-HCl reagent is employed in the quantitative estimation. At present trypsin hydrolysis of proteins is the most satisfactory procedure for the estimation of tryptophane in proteins. By the method here developed we find 1.26 per cent in casein, 0.53 per cent in U.S.P. pancreatin, 0.19 per cent in zein, and 0.12 per cent in gelatin. The value found for casein agrees very favorably with the assumptions that the molecular weight of casein is of the order of 16,000, and that 1 molecule of tryptophane is present. The low values for zein and gelatin suggest molecular weights of the order of 106,000 and 120,000, respectively, or that the tryptophane is present as an impurity in the form of another protein. Pure tryptophane, or tryptophane and monoamino acids, when incubated with trypsin, is recovered quantitatively by the vanillin-HCl method, but when incubated therewith in the presence of the diamino acid fraction or glucosamine a loss of 10 to 20 per cent is observed. Without incubation this loss is not observed.

ON THE RELATION OF THE THYROID TO THE EFFECTS OF INSULIN.

By AARON BODANSKY.

(From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca.)

The author has reported the typical time relations of the blood sugar curves obtained after intravenous injection of insulin in normal and thyroidectomized sheep, and in normal human subjects. His findings on the effect of thyroidectomy on the reaction to insulin have been confirmed by Ducheneau and Burn and Marks.

In an effort to establish more rigorous quantitative criteria for the reaction of the athyroid, hypothyroid, and hyperthyroid, as well as of the normal subject, to intravenous injections of insulin, the author has attempted to ascertain the conditions under which practically constant blood sugar is obtained in sheep, and a practically constant response to injections of insulin, and has repeated his experiments under these conditions.

FURTHER STUDIES ON THE EFFECT OF FINE GRINDING UPON STARCH GRAINS.

By C. L. ALSBERG and E. E. PERRY.

(From the Food Research Institute and the Department of Chemistry, Stanford University, California.)

We have shown recently that cold water extracts considerable material, giving a blue color with iodine from starch grains ground in a pebble mill until the individual grains are broken or battered, and that a suspension of such ground starch in water is incapable of gelatinizing or yielding a paste when heated (except in concentrations of 10 to 15 per cent by weight). It can now be reported that such battered starch grains are still birefringent, though they no longer show the black cross under the polarizing microscope. They swell instantaneously when they come in contact with cold water and to a considerable degree disperse themselves in it. They stain instantaneously in aqueous solutions of a number of dyestuffs as well as being dispersed in them. Uninjured starch grains are stained with the greatest difficulty. Solutions prepared by extracting ground starch in water at room temperature deposit on standing a flocculent precipitate insoluble in cold water. However, not all the soluble material is deposited. The solution after weeks of standing still contains about 0.5 per cent of material which colors blue with iodine. It is precipitated from its solutions by alcohol and the precipitate can be redissolved in cold water if it has not been permitted to become dry.

A MODIFICATION OF THE METHOD OF PREPARING GLIADIN.

By D. B. DILL and C. L. ALSBERG.

(*From the Food Research Institute and the Department of Chemistry, Stanford University, California.*)

The modification consists essentially of two procedures. The first is that after the first alcoholic extract of gluten has been concentrated to a thick sirup under diminished pressure and the gliadin has been precipitated with water containing a little sodium chloride, it is redissolved in warm 70 per cent alcohol and the solution is allowed to stand in the refrigerator overnight. In this manner a gelatinous mass—impure gliadin—settles out which carries down with it nearly all the contaminating lipoid material. This is otherwise very difficult to remove and interferes with clear filtration. The gliadin is precipitated with strong alcohol out of the clear filtrate after evaporation to small volume, whereupon it is again subjected repeatedly to the processes above described. In the final precipitation with water lithium chloride is used instead of sodium chloride; and this is the second modification of Osborne's method of preparation proposed in this paper. Lithium chloride has the advantage over sodium chloride where precipitation with alcohol is unsatisfactory unless an electrolyte be present, that it is freely soluble in alcohol and ether and may thus be more easily removed again.

Five preparations were made from three different flours. Their nitrogen contents (Kjeldahl) on the dry basis are 17.55, 17.53, 17.53, 17.56, and 17.49 per cent. Their ash contents are 0.06, 0.11, 0 07, 0.07, and 0.08 per cent. Loeb, C. R. Smith, and others consider gelatin prepared by washing at the isoelectric point and still containing nearly 0.1 per cent ash, as ash-free.

THE SPECIFIC ROTATION OF GLIADIN IN EtOH-H_2O SOLUTIONS.

BY D. B. DILL AND C. L. ALSBERG.

(From the Food Research Institute and the Department of Chemistry, Stanford University, California.)

We have determined the specific rotation of five samples of pure gliadin, obtained from three different flours. $[\alpha]_D^{20}$ for gliadin dissolved in 50 per cent alcohol was found to be −90.0°; in 60 per cent alcohol, −91.0°; in 70 per cent alcohol, −90.0°; in 80 per cent alcohol, −90.2°. Freshly prepared solutions were found to have a specific rotation about one degree greater (arithmetically) than those noted above. Our solutions were prepared by weighing the gliadin in 25 cc. volumetric flasks, adding 20 cc. of alcohol, and warming at 40° until solution was complete. The flasks were then cooled to 20°, the contents made up to volume, and polarizations made. From 12 to 48 hours later second readings were made with the results indicated above. It is believed that there is some lag in the return of the specific rotation of gliadin solutions to their normal value as they are cooled from 40° to 20°.

$[\alpha]_D^{30}$ for gliadin in 50 per cent alcohol is −91.4°; in 60 per cent alcohol, −93.2°; and in 70 per cent alcohol, −91.4°. $[\alpha]_D^{40}$ for gliadin in 50 per cent alcohol is −94.2°; in 60 per cent alcohol, −95.8°; and in 70 per cent alcohol, −93.4°. As these solutions were warmed from 20° to 30° and from 30° to 40°, the values for $[\alpha]_D^{30}$ and $[\alpha]_D^{40}$ were reached immediately and did not change after 30 minutes at each of these temperatures. Upon cooling each of these solutions from 40° to 30° the previous value for 30° was immediately attained except in the case of the solution in 70 per cent alcohol. Upon cooling from 30° to 20°, some time elapsed before the original value for $[\alpha]_D^{20}$ was attained.

STUDIES ON GLYCOLYSIS.

BY SERGIUS MORGULIS AND OTAKAR BARKUS.

(From the Department of Biochemistry, University of Nebraska, Omaha.)

In view of the importance attributed by the Embden school to lactacidogen as an intermediate phase in carbohydrate metabolism, we studied the changes in inorganic phosphorus of blood undergoing glycolysis. It is, of course, known that the disappearance of the blood sugar is paralleled by an accumulation of lactic acid in the blood. If lactacidogen were, therefore, an intermediate step in this transformation, one would likewise expect to find a gradual rise in the inorganic phosphorus. Indeed, it is claimed by some that this is what actually occurs in the course of glycolysis. We performed experiments with bloods from various animals, which have been undergoing glycolysis at 33°C., and we followed the changes in sugar, lactic acid, and inorganic phosphorus at different intervals during the process. In these studies we found that the inorganic P invariably remained unchanged even when glycolysis has progressed very far and there has been a great accumulation of lactic acid in the system. This, however, is only true for experiments where there has been no hemolysis. Even a trace of hemolysis will cause an appreciable rise in the inorganic P value and, as the hemolysis becomes more extensive, the inorganic P value likewise increases manifold. We are led, therefore, to the conclusion that the formation of lactic acid resulting from glycolysis of blood is not associated with any alteration in the inorganic P content provided there is no hemolysis occurring at the same time. Evidence which has been offered to the contrary is unquestionably due to the fact that the effect upon the inorganic P produced by hemolysis has not been properly considered.

THE MAXIMUM OF HUMAN POWER, AND THE FUEL OF MUSCULAR WORK, FROM OBSERVATIONS ON THE OLYMPIC CHAMPIONSHIP CREW OF 1924.

By YANDELL HENDERSON and HOWARD W. HAGGARD.

(*From the Department of Applied Physiology, Yale University, New Haven.*)

Rowing in a racing shell with sliding seats probably allows a nearer approach to maximal work, by more nearly all the muscles of the body, than any other form of athletics. The eight men who rowed in the Yale University boat in 1924 demonstrated in a series of races, ranging from 1¼ up to 4 miles, that for all these distances they were able to lead any other crew in their own country. After winning the right to represent America in the Olympic games at Paris, they won in the trial heats and led by several lengths in the final race on the Seine, winning from crews from all parts of the world and establishing a world's record for the 2,000 meter course.

During the season of training from January to June, 1924, we were fortunately able to make from time to time on five of these men determinations of the respiratory exchange and quotient, the oxygen consumption and deficit, the CO_2 output, etc. The external work was also determined by means of a rowing machine set up in the laboratory and arranged as an ergometer. Later a determination of the power necessary to drive a racing boat at various speeds was obtained by towing it by means of a power boat with the tow line fastened to a spring balance. The figures from the draw bar pull multiplied by the speed in feet per minute give the absolute net work which the crew had to do. An additional 25 per cent of external work was assumed in order to cover the energy expended in moving the slide and oar in returning to the stroke position, and was added to the results from the rowing machine and draw bar pull methods.

The data from these three methods were in general in fair agreement. They indicate that the maximal power exerted is from 0.45 to 0.55 horse power per man, or expressed in the heat equivalents, 4.8 to 5.9 calories per minute, with a total energy expenditure of 19 to 29 calories per minute, or thirteen to twenty times the basal rate. The power expressed by the smaller of each of these pairs of figures is that maintained, and is therefore approximately the maximum that a man can maintain, for 22 minutes during a 4 mile race; while the higher figures are applicable to the more intense exertion and greater speed, which are also maximal for about 6 minutes in races of about 1¼ miles or 2,000 meters. The corresponding figures for the volume of oxygen consumed per minute are 3.5 and 4 liters; the latter figure is about the limit of the transporting capacity of the lungs, blood, and heart. An oarsman exerts a power, which exceeds by 30 to 60 per cent that afforded by the oxygen simultaneously absorbed; he thus draws heavily on his credit, and incurs oxygen deficits of 4 to 8 liters or more and these deficits are repaid by the high rate of oxygen absorption for a time after the work is ended. This is in accord with A. V. Hill's conception.

The most significant result of these observations is the evidence, which they afford, in general agreement with Krogh and Lindhard, but in disaccord with the Hill-Meyerhof conception in its original form, that in whatever proportion fat and sugar are being burned during rest just before the exercise, they are burned in nearly the same proportion to produce the energy for doing work and for recovery. Thus it is found that in these oarsmen the respiratory quotients for the work and recovery periods combined were approximately the same as those during the rest before the work. In one experiment the man had missed his breakfast and eaten nothing for 18 hours; yet he made an intense exertion, although rather disadvantageously, on a combustion almost entirely of fat from his own body. His respiratory quotient during rest was 0.75, during 3 minutes of rowing on the machine it was 0.72, and during 10 minutes rest afterward 0.73. The work done was equivalent to 6 calories per minute, the total energy expenditure 29 calories per minute; this man's weight was 180 pounds (82 kilos) and his height 6 feet, 4 inches (193 cm.). In general the observations show that much more than half the energy expended by these athletes in muscular work is drawn from fat, and much less than half from sugar. A much larger proportion of carbohydrates would probably be advantageous.

In contrast to the effects of great exertion on untrained men, there was in the members of this crew only a slight overbreathing, or sometimes practically none at all, with a correspondingly slight blowing off of CO_2 during work or afterward. Apparently some of the phenomena, especially the blowing off of CO_2 and the high respiratory quotient during and immediately after intense exertion, which are commonly explained as in accord with the Hill-Meyerhof conception of muscular contraction, are due to the stimulation of the respiratory nervous regulation by oxygen deficiency in the arterial blood rather than to displacement of carbonic acid from the blood carbonates by lactic acid.

FURTHER DATA ON THE METABOLISM OF DEPANCREATIZED DOGS KEPT ALIVE WITH INSULIN.

By I. L. CHAIKOFF, J. J. R. MACLEOD, and J. MARKOWITZ.

(From the Department of Physiology, University of Toronto, Toronto, Canada.)

By daily feeding with raw pancreas (50 gm.) along with flesh (200 to 400 gm.) and cane-sugar (up to 50 gm.), and the injection twice daily of about 16 clinical units of insulin, dogs weighing approximately 8 to 10 kilos can be kept in a perfect condition of bodily nutrition. One animal is still living after 15 months on this diet, and another after 9 months. Since none of the symptoms of hepatic breakdown, previously reported from this laboratory (Allan, Bowie, Macleod, and Robinson) as occurring in the case of depancreatized animals fed without raw pancreas, have supervened, it is concluded that the presence of something derived from the pancreas, either its digestive ferments or some internal secretion besides insulin, is necessary for the maintenance of normal metabolism. It is possible that the absorption from the intestine of toxic breakdown products of protein is responsible for the damage to the liver when no trypsin is present.

Various studies have been made of the chemical changes which occur in the blood and urine following discontinuance of insulin in these animals, for several days, and also of the changes produced by again giving this hormone. The main results show a remarkable correspondence in the rate at which changes occur in sugar, in acetone bodies, and in the phosphoric acid of the blood. All three come down at an equal rate after injecting insulin, but the phosphoric acid starts to return to the original level more quickly than the other two. No constant changes have been observed in the percentage of fat in the blood within several hours of the injection of insulin. The percentage amounts of ketone bodies, of sugar, and of fat are higher 3 to 5 days after discontinuing insulin in a fat depancreatized dog than in one that is thin.

CHEMICAL CHANGE IN FISH MUSCLE DURING RIGOR MORTIS.

By C. C. BENSON.

(From the Department of Food Chemistry, University of Toronto, and Atlantic Biological Station, St. Andrews, New Brunswick, Canada.)

It has been noted by von Fürth that the juice squeezed out from fish muscle contains considerable amounts of the protein which he calls "soluble myogen-fibrin" and, by a method which he describes, it is possible to determine its amount fairly quickly.

When large fishes were available, the tail parts were cut off and set in an apparatus to measure the amount of rigidity following death, and the remaining parts of these fishes were used to obtain the muscle juice; or two fishes of similar size and weight were used—one for the measurement of stiffness and the other, for chemical study.

It was found that, when the fishes were soft, before rigor set in, the amount of soluble myogen fibrin was less than during rigidity and that the amount decreased as the stiffness passed off. The amount of this protein in the muscle juice is thus related to the condition of the muscle in regard to rigidity.

THE RELATIVE REDUCING POWER OF SOME COMMON SUGARS.

By A. W. ROWE and B. S. WIENER.

(From the Department of Chemistry, Evans Memorial, Boston.)

It has long been recognized that the reduction velocities of the simple sugars exhibit wide variations. Blood sugar methods are based upon incomplete reductions and consequently are most affected. Blood sugar curves produced by test meals of sugars other then glucose are never accurate and may be seriously in error. The relative reducing values compared with glucose as 100 are given in Table I.

TABLE I.

Method	Folin-Wu.		Lewis-Benedict.
Time	6 min.	12 min.	10 min.
Glucose	100	105	100
Levulose	90	90	99
Galactose	77	88	85
Mannose	55	92	100
Lactose	45	59	76
Maltose	40	50	82

Mixtures of the several sugars dissolved both in water and in blood plasma showed that the relative reducing powers depend upon the coefficient of each sugar and their relative proportions. With the disaccharides, hydrolysis is a possible factor, particularly in the picric acid method.

The application of the several coefficients to the calculation of approximate blood sugar levels is pointed out.

DIETARY REQUIREMENTS FOR REPRODUCTION.

V. THE DISTRIBUTION OF THE REPRODUCTIVE DIETARY COMPLEX (VITAMIN E) IN VARIOUS VEGETABLE OILS.

By BARNETT SURE.

(From the Department of Agricultural Chemistry, Agricultural Experiment Station, University of Arkansas, Fayetteville.)

From work thus far completed the vegetable oils may be tentatively divided into three groups.

1. Those that do not even produce fertility. In this group belong commercial linseed, cocoanut, and sesame oils.

2. (*a*) Those oils which will produce fertility but fail in lactation. In this group belong commercial olive oil and the oil of the peach kernel; also soy bean and peanut oils directly expressed. (*b*) Those which produce fertility and are partly successful in lactation. In this group belongs commercial cottonseed oil.

3. Those oils which are potent both in fertility and lactation. In this group belong wheat germ, hemp-seed, and yellow corn oils, prepared by extraction with ether, acetone, or benzene.

Experiments are in progress to determine whether the reproductive complex is made up of two vitamins, one necessary for fertility, and one essential for lactation.

THE PRESENCE OF TREHALOSE IN BAKERS' YEAST.

By ELIZABETH M. KOCH AND F. C. KOCH.

A QUANTITATIVE PEPSIN METHOD BASED UPON REFRACTIVE INDEX AND MICRO KJELDAHL ESTIMATIONS.

By T. L. MEEKIN.

A METHOD FOR PURIFYING INSULIN.

By N. R. BLATHERWICK AND L. C. MAXWELL.

THE DESTRUCTION OF URIC ACID IN PERFUSION EXPERIMENTS.

By OTTO FOLIN, CECIL DRINKER, AND SIDNEY BLISS.

THE SITE OF AMMONIA FORMATION.

By OTTO FOLIN AND SIDNEY BLISS.

THE INFLUENCE OF THE HORMONE OF THE OVARIAN FOLLICLE ON THE BASAL METABOLISM OF THE RAT.

By EDWARD A. DOISY AND J. O. RALLS.

SOME RECENTLY INTRODUCED MICRO QUANTITATIVE METHODS AND THEIR VALUE.

By A. B. MACALLUM.

ABSTRACT OF THE EXECUTIVE PROCEEDINGS OF THE NINETEENTH ANNUAL MEETING.

New Members: Cyril Berkeley, Mary L. Caldwell, E. P. Clark, F. A. Csonka, H. J. Deuel, Jr., L. T. Fairhall, R. M. Ferry, R. A. Gortner, G. Hunter, Cornelia Kennedy, Grace Macleod, V. E. Nelson, I. Neuwirth, W. A. Perlzweig, Atherton Seidell, A. W. Thomas.

Clinical Members: A. Koehler, F. W. Schlutz.

Deceased Members: Jacques Loeb, Benjamin S. Neuhausen.

Officers for the Year 1925: *President,* Henry C. Sherman; *Vice-President,* Edward C. Kendall; *Secretary,* D. Wright Wilson; *Treasurer,* Walter R. Bloor; *Additional Members of the Council,* Otto Folin; Lafayette B. Mendel; Philip A. Shaffer; *Nominating Committee,* H. C. Bradley, J. B. Collip, E. A. Doisy, F. S. Hammett, F. C. Koch, H. B. Lewis, E. V. McCollum, C. P. Sherwin, D. D. Van Slyke.

Attendance: Ninety-six members of the Society attended the meeting.

D. Wright Wilson,
Secretary.

Jacques Loeb.

The death of Jacques Loeb in the full tide of productive activity has robbed our Society of a distinguished and illustrious member, and the world of science and culture of an outstanding leader.

In our Society Jacques Loeb was one of the older members and yet of the most modern. Every historic epoch makes a special demand on every branch of human activity and on every branch of science. The man whose mind is the keenest, whose vision is clearest, and whose imagination is the strongest, receives the message first and becomes the leader: such was Loeb.

The generation of physiologists preceding Loeb had introduced into biology the methods and doctrines of physics as a means of disclosing the mechanisms of life phenomena. Helmholtz, du Bois-Reymond, Julius Sachs, Pfeffer, and others were the leaders of that period. However, those who were concerned with animal physiology concentrated their attention rather on specific functions than on the general properties of living matter.

Jacques Loeb's keen vision early revealed to him that the most fundamental concern of the biologist is the nature of living matter. It soon became evident to him that in its physical state living matter presented a heterogeneous system and that the properties of such systems could be revealed best by the methods of the then new science of physical chemistry. In the researches inspired by this vision, he developed a new type of general biology. As his work progressed, Loeb realized that certain chapters of physical chemistry which were of special importance to the biologist were incomplete. He successfully undertook their development and added to his unique leadership in biology an outstanding position among chemists.

Those who came into personal contact with Jacques Loeb will always remember the inspiration he conveyed through his boundless devotion to Science, the height of his idealism, the charm of his personality, his sympathies with the younger, but even more important is the message he bequeathed to the workers in all the various branches of biology. This message reads: "It is not sufficient for the biologist to follow the guidance of the more fundamental sciences, physics and chemistry, but it is imperative for him to acquire the equipment which is necessary to develop those chapters of physics and chemistry which are needed to clarify the mechanism of the phenomena of life." The life of Jacques Loeb was the most sustained expression of this thought, and as such it will remain in the memory of biologists of the times to come.

P. A. Levene,
Victor C. Myers,
Donald D. Van Slyke,
Committee.

Benjamin S. Neuhausen.

Benjamin S. Neuhausen died in August, 1923, at the beginning of his scientific career. He received the degree of Doctor of Philosophy from the Johns Hopkins University in June, 1921, and was associated from that time until his death with the Department of Physiology of the Johns Hopkins Medical School. In this short space of two years he published several papers. His main efforts were directed to the problem of the determination of concentrations of various ions in biological fluids—the concentration of the sodium, calcium, chloride ions, etc. The electrometric method, now so well known for the estimation of hydrogen ion concentration was used, and a preliminary report of the results published. With these results, however, Neuhausen was not satisfied and was engaged in improving the methods used.

He was elected a member of the American Society of Biological Chemists at the meeting preceding his death, and did not have the opportunity of attending a meeting. Neuhausen was a quiet, reserved, and diligent worker, and in his passing Science has lost one who gave promise of a brilliant future.

E. K. Marshall, Jr.,
D. Wright Wilson,
Committee.

PROCEEDINGS

OF THE

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

TWENTIETH ANNUAL MEETING

Cleveland, Ohio

December 28-30, 1925

ABSTRACTS OF PAPERS
ABSTRACT OF EXECUTIVE PROCEEDINGS

Volume VII
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For the List of Members and the Constitution and the By-Laws of the Society see the 1926 Register of the Federation of American Societies for Experimental Biology, comprising the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, and the American Society for Experimental Pathology.

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CAN OTHER IMIDAZOLES REPLACE HISTIDINE IN THE DIET FOR PURPOSES OF GROWTH?

By GERALD J. COX and WILLIAM C. ROSE.

(*From the Laboratory of Physiological Chemistry, University of Illinois, Urbana.*)

Studies have been made of the influence upon growth of synthetic compounds containing the imidazole ring. For this purpose the substances in question were added to a basal diet in which the nitrogen was supplied in the form of completely hydrolyzed casein from which histidine had been removed by precipitation with silver sulfate and barium hydroxide. No influence upon the growth of rats was observed following the inclusion in the ration of imidazole, 4-methyl imidazole, 4-hydroxylmethyl imidazole, 4-imidazole formaldehyde, 4-imidazole carboxylic acid, 4-imidazole acetic acid, 4-imidazole propionic acid, or 4-imidazole acrylic (urocanic) acid. The inclusion in the diet of one or two equivalents of either of the above substances entirely failed to inhibit the loss in weight occasioned by the deficiency of histidine.

On the other hand, the addition of *dl*-4-imidazole lactic acid to the histidine-deficient diet caused an immediate resumption of growth, but at a rate slightly slower than that induced by the equivalent quantity of histidine. The above results constitute the first successful attempt to replace an "indispensable" amino acid of the diet by a non-amino compound.

THE CHEMICAL CONFIGURATION OF THYROXIN AND ITS MODE OF ACTION IN THE TISSUES.

By EDWARD C. KENDALL.

(*From the Section of Biochemistry, Mayo Foundation, Rochester, Minnesota.*)

The recent work of Hicks at Cambridge, England, has demonstrated by means of the absorption of the ultra-violet spectrum the presence of the indole nucleus in thyroxin. Furthermore, he has shown that the pyrrolidone ring is open or closed under conditions similar to those in which I have shown it to be open or closed by chemical tests. The only remaining problem, therefore, with the configuration of thyroxin lies in the position of the 3 atoms of iodine and the 2 atoms of hydrogen. By synthesis, we have eliminated the possibility of one of the double bonds being in the pyrrolidone ring and also by synthesis, we have proved the stability of the halogens in the benzene ring when they are in positions 4, 5, and 6.

The difficult problem in the synthesis of these compounds has been to add the third halogen. This has recently been accomplished and 3 atoms of bromine have been added to the benzene ring in positions 4, 5, 6. When the 3 atoms ot bromine are added to the unreduced benzene ring in positions 4, 5, and 6, a compound is produced whose chemical properties are not similar to those of thyroxin. Attempts were therefore made to reduce the benzene ring and add 2 atoms of hydrogen. This cannot be brought about by reduction but further investigation showed the benzene ring is sensitive to oxidation. The tribromo compound with bromine atoms in positions 4, 5, 6 will add 2 more atoms of bromine on positions 5 and 6, forming a pentabromo derivative. This substance is surprisingly stable and it is reduced with difficulty to a tribromo 4, 5, 6 leaving 2 atoms of hydrogen, one on 5 and one on 6. This substance has the formula assigned to thyroxin except there are 3 atoms of bromine in the positions occupied by the 3 atoms of iodine. The chemical properties of this compound are strikingly similar to those of thyroxin. It is stable to reduction and to strong alkali, exists with the pyrrolidone ring open or closed, and gives the unique color reaction with nitrous acid and an alkali.

The physical and chemical properties of the tribromo thyroxin confirm as strongly as anything can the correctness of the formula assigned to thyroxin.

By means of a study of the oxidation-reduction potentials of a number of these synthetic compounds which are closely related to thyroxin, it can be shown that the hydrogen on No. 7 carbon is easily oxidized. Dibromoindolephenol in the complete absence of oxygen is capable of oxidizing any of these derivatives. The fully reduced and fully oxidized forms have very little effect on the platinum electrode. The partially oxidized form however develops a strong reducing potential and indicates that there exists an intermediate compound possibly possessing the properties of a free radical, which is the actively functioning form of this series of compounds. Further work has shown that epinephrine behaves in an entirely similar manner. Neither its fully reduced or fully oxidized form possesses the reactivity of the intermediate partially oxidized molecule. Also cysteine-cystine and oxidized and reduced glutathione are other members of this group of substances. The fully oxidized and reduced forms of these compounds are not in equilibrium with each other but they are in equilibrium with the partially oxidized or free radical forms of the substances. Thyroxin, epinephrine, and glutathione are members of a group of substances which furnish to the animal organism an active chemical grouping. They establish the intensity of the oxidation-reduction process occurring within the cells, and produce a poising effect maintaining the intensity of oxidation and reduction within narrow limits.

SOME PROPERTIES OF THE HORMONE OF THE LIQUOR FOLLICULI.

By J. O. RALLS, C. N. JORDAN, C. T. HEUSINKVELD, and EDWARD A. DOISY.

(From the Department of Biological Chemistry, St. Louis University School of Medicine, St. Louis.)

Liquor folliculi has been preserved with two volumes of 95 per cent alcohol for a period of 1½ years without suffering loss of potency. The stability of highly purified products seems to be remarkably less than that of crude products.

Our best method of preparation (to be published soon) yields a product of which approximately 0.04 mg. is one rat unit. The method is based merely upon the differential solubility of the hormone in various common organic solvents.

Data were presented upon the effect of hydrolysis, hydrogenation, acetylation, benzoylation, and bromination upon the activity of the hormone. The percentages of carbon, hydrogen, nitrogen, and phosphorus were determined on our best preparation of which 0.04 of a mg. was one rat unit.

NOTES ON THE CHEMISTRY AND PHYSIOLOGY OF THE ANTIPRESSOR FRACTION FROM HEPATIC TISSUE.

By A. A. JAMES, N. B. LAUGHTON, and A. BRUCE MACALLUM.

(From the Departments of Biochemistry and Physiology, University of Western Ontario Medical School, London, Ontario, Canada.)

We have obtained from absolutely fresh liver of herbivores, omnivores, and carnivores a substance giving a sustained fall in blood pressure. The yield from herbivores does not seem so consistent.

The fresh washed liver is well frozen and thawed two or three times in a slightly acid medium. The proteins are removed by treatment with alcohol up to 75 per cent at the isoelectric point and the whole allowed to stand 24 hours. After removal of the alcohol the active principle can be precipitated by phosphotungstic acid or acetone. In the case of the former the phosphotungstates are decomposed with baryta in a water-acetone suspension, taken to dryness, purified, and finally shaken in aqueous solution with ether. The ether contains a water-soluble substance which causes a sustained fall in blood pressure, gives no biuret reaction, no indication of histamine with *p*-diazobenzene sulfonic acid, or crystals of choline with iodine.

Acetone precipitates from the extract a pressor substance of unknown constitution which can be removed by shaking with Lloyd's reagent in ½ per cent acetic acid.

In clinical hypertension the preparation has a relatively more profound effect than on the normal mammalian blood pressure.

The site of action is not on the capillaries as in the case of histamine. There is no peripheral dilatation. In the mesenteric blood vessels there can be seen venous engorgement with slowing of the blood stream in veins and arterioles. The capillaries show no dilatation.

KETOSIS IN THE RAT.

By ARTHUR H. SMITH and HAROLD LEVINE.

(From the Laboratory of Physiological Chemistry, Yale University, New Haven.)

Normal growth from 30 to 180 gm. of body weight can be obtained in rats on a high fat diet containing 86 per cent of the total calories as fat calories. The utilization of fat in these experiments ranged from 96 to 99 per cent.

In metabolism experiments, no essential difference in the urinary output of "acetone bodies" was evident whether the diet was composed largely of fat, protein, or carbohydrate or consisted of a balanced mixture of these dietary essentials. On changing suddenly from a high carbohydrate or high protein diet to a high fat régime, no change in the level of the "acetone bodies" excretion was noticeable. The quantity of "acetone bodies" excreted by the normal rat is independent of the nature of the diet and varies in most animals from 1 to 3 mg. (expressed as acetone) per day. Fasting produced no appreciable ketosis in the rat contrasted to the human species.

The results mentioned above seem to point to a type of fat-carbohydrate metabolism in the rat which is different from that in man. Rats on any of the above mentioned régimes put out a rather constant proportion of beta-hydroxybutyric acid (expressed as acetone); *i.e.*, approximately 70 per cent of the "total acetone bodies." When sodium bicarbonate constituted 12 per cent of a high fat diet, an immediate rise in the output of "acetone bodies" was observed. On changing back to the high fat diet without the alkali, the "acetone bodies" excreted promptly fell to the former low level.

THE CONDUCTIVITY OF ERYTHROCYTES TO ELECTRIC CURRENTS OF HIGH AND LOW FREQUENCY.

By J. F. McCLENDON.

(*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis.*)

Frequencies from 120 to 4000 cycles are obtained from a Vreeland oscillator 10 meters from the bridge, high frequency currents up to a million, from an electron tube oscillator with toroidal coils 5 meters from the bridge. One corner of the bridge is grounded. The bridge consists of a meter platinum wire with sliding contact connected to the ground by means of a wire dipping in a mercury trough, and two conductivity cells made exactly alike with gold plates 25 mm. in diameter and 10 mm. apart. Conductivity of the blood is compared with that of a KCl solution of about the same conductivity. The reactance is balanced by means of decade toroidal coils connected in series with the conductivity cell. All parts of the system have an electrostatic shield placed at least 3 inches from the conductors, and the conductivity cells are placed in an air thermostat. A special device keeps the blood corpuscles stirred up. A curve was shown of the ratio of conductivity of serum to conductivity of blood at different corpuscle volumes up to 99 per cent. Corpuscle volumes are determined by means of a hematocrit mounted on the shaft of a Dumore high speed electric motor. The resistance of serum is the same at a thousand and million cycles within 1 per cent. The resistance of corpuscles at 1000 cycles is five to eight times as great as at a million cycles. Laking does not change resistance of corpuscles to a million cycles more than 7 per cent but increases that to 1000 cycles to equal that to a million (within 20 per cent). The resistance of sugar-washed corpuscles to 1000 cycles is 50 times as great as of serum, but to a million cycles is eight times as great. The curve of R_{b/R_s} against cell volume, at 1000 cycles approaches Clerk Maxwell's formula assuming the corpuscles are insulators, while that to a million cycles diverges greatly but approaches that calculated, assuming less corpuscle resistance. As the electrolyte is diluted, the electric double layer becomes thicker. The resistance of the corpuscle interior is about equal to that of a 0.01 N KCl solution.

THE PRECIPITATION OF UREASE BY LEAD ACETATE.

By JAMES B. SUMNER.

(*From the Departments of Physiology and Biochemistry, Cornell University Medical College, Ithaca.*)

Urease is entirely precipitated from solution by a slight excess of neutral lead acetate. If the precipitate is centrifuged and stirred with sodium sulfate solution, much of the protein, but no urease, is liberated. After washing four times with sodium sulfate, the urease can be largely recovered by extraction with oxalate-phosphate solution. This urease is not so pure, in respect to its protein content, as that prepared by the cooling process.[1] It appears to contain a globulin different from canavalin, concanavalin A and concanavalin B. Upon dialyzing there is no precipitate. Dialysis against thousandth normal acetic acid causes the solution to become opalescent. The addition of sodium chloride now causes the precipitation of insoluble urease.

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[1] Sumner, J. B., and Graham, V. A., *Proc. Soc. Exp. Biol. and Med.*, 1925, xxii, 504.

Insoluble urease contains no iron and is entirely free from pectase, which is present in the jack bean in large amount.

STUDIES ON GLUTELINS.

By D. BREESE JONES and FRANK A. CSONKA.

(From the Protein Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.)

It was noted in certain solubility studies that sodium chloride solution may have a denaturing effect on proteins. Therefore, it is possible that during the extraction of seeds and raw materials with saline solutions the albumins and globulins become more or less denatured, and are thus rendered partly insoluble, the denatured portion remaining in the extracted residue. Since glutelins are generally prepared from residues that have been exhaustively extracted with salt solutions, the application of alkali as a solvent for the glutelins will dissolve these denatured proteins, and consequently the glutelin preparation obtained on neutralization of the alkaline extract with acid will consist of a mixture of proteins and not of glutelin alone.

It was found that both wheat gliadin and wheat glutenin are precipitable from their alkaline solutions by addition of very small quantities of ammonium sulfate. We separated two glutenin fractions. The first fraction was obtained by making the alkaline solution 0.02 to 0.04 saturated with ammonium sulfate, and the second fraction precipitated at 0.1 to 0.15 of saturation. Both preparations had an isoelectric point of 6.45, which was identical with that found for gliadin. Work is under way for a further characterization of these two fractions.

The filtrate from the second fraction, on further addition of ammonium sulfate to 0.2 to 0.3 of saturation, yielded a relatively small quantity of another precipitate, having an isoelectric point of 5.45. Both the isoelectric point and degree of saturation with ammonium sulfate at which this fraction was precipitated come well within the range of these constants for globulins.

ON BIOCHEMICAL COLOR REACTIONS WITH BENZIDINE, *p*-PHENYLENE DIAMINE, ETC.

BY W. MANSFIELD CLARK, BARNETT COHEN, AND H. D. GIBBS.

(From the Division of Chemistry, Hygienic Laboratory, United States Public Health Service, Washington.)

The electromotive conduct of tolidine, *p*-amino dimethyl aniline, and similar diamines of the phenylene diamine and benzidine series has permitted: (1) The determination of basic dissociation constants of the diamine (reductant). (2) The determination of basic dissociation constants of the diimide (oxidant). (3) The "normal" oxidation-reduction potential. (4) The degree of association of those species of oxidant and reductant which form the so called meriquinone or colored compound used in tests of oxidative action.

The quantitative data have provided an outline of the very complicated set of equilibria involved in these color tests, and have shown the impracticability of attempting to make benzidine and similar reagents useful in precise tests of oxidizing intensity. Tentatively, however, the data when combined with reports in the literature, concerning the ability of cell suspensions to oxidize these diamines, indicate a very low level of oxidizing intensity.

THE OXIDATION AND REDUCTION POTENTIALS OF SOME COMPOUNDS CLOSELY RELATED TO THYROXIN.

BY JOHN M. ORT.

(From the Section of Biochemistry, Mayo Foundation, Rochester, Minnesota.)

The oxidation-reduction potentials and chemical activity of 2-oxodihydroindole-3-propionic acid and some of its halogen derivatives have been determined. The results show that in both the fully reduced and fully oxidized forms these substances have very little effect on a platinum electrode. When the reduced form is partially oxidized an intermediate compound is produced which has a marked reducing potential.

The chemical activity of these compounds closely parallels these findings. The reduced form of the above compounds reduces dibromoindophenol slowly. After partial reduction of the dibromoindophenol, the action becomes more rapid and continues to the complete reduction of this dye. The addition of 2 atoms of bromine or of iodine to the indole nucleus of these compounds markedly increases the rate of reduction of dibromoindophenol but does not change the intensity of the reducing power of the intermediate or active state of the molecule.

DIFFERENTIATING REACTIONS FOR CYSTEINE, CYSTINE, AND GLUTATHIONE.

By M. X. SULLIVAN.

(From the Division of Chemistry, Hygienic Laboratory, United States Public Health Service, Washington.)

Cysteine and reduced glutathione give a purple-red color with sodium nitroprusside and ammonium hydroxide. Cystine and oxidized glutathione do not. Addition of sodium cyanide makes cystine and oxidized glutathione give the purple nitroprusside reaction. Cysteine gives a red color with 1.2 naphthoquinone-4-sodium sulfonate and sodium sulfite in alkaline solution. Cystine does not, but will give the red color if treated with NaCN before addition of the naphthoquinone. Glutathione does not give the red color with the naphthoquinone, either with or without treatment with sodium cyanide. Hydrolyzed glutathione gives the naphthoquinone reaction as given by cystine. By proper treatment with these reagents, with and without use of sodium cyanide, cysteine, cystine, and glutathione can be determined quantitatively in pure solution and roughly quantitatively at present in mixtures of the four compounds, cysteine, cystine, reduced glutathione, and oxidized glutathione.

A NEW ACID-FORMING ENZYME IN GASTRIC AND OTHER TISSUES, AND ITS POSSIBLE SIGNIFICANCE IN THE GASTRIC HYDROCHLORIC ACID MECHANISM.

By MARTIN E. HANKE.

(From the Laboratory of Physiological Chemistry, University of Chicago, Chicago.)

A new theory has been developed for the chemical mechanism of the formation of hydrochloric acid in the gastric juice, which assumes essentially the hydrolysis of an alkyl chloride by a specific enzyme activity with the production of the corresponding alkyl alcohol and hydrochloric acid. In testing this theory experimentally the following results have been obtained.

1. When fresh aqueous gastric tissue extract is incubated with any one of a number of chloride esters, acidity is produced, which is a direct function of the amount of tissue extract and the amount of chloride ester used.

2. The "chloride ester activity" is quite distinct from the ordinary gastric and other tissue lipases.

3. Using glycerol *αα*-dichlorohydrin, $ClCH_2CHOHCH_2Cl$, in M/20 solution (N/10 chloride) and a 10 per cent fresh gastric tissue extract, about one-tenth of an equivalent amount of acid is formed after incubating for 15 hours at 37°C., *i.e.* N/100 acid. Using glycerol dichlorohydrin as standard, other chloride esters may be arranged in the order of their relative activities, thus:

Glycerol dichlorohydrin	100
" monochlorohydrin	28
Monochloroacetic acid	28
Dichloroacetic "	16
Trichloroacetic "	0
Chloroform	0

That the activity is a function of the chemical nature of the chloride ester is evident. It seems particularly significant that the monochlorohydrin and monochloroacetic acid *always* give the same amount of activity, and that they are both the same type of chloride ester, *i.e.* a monochloro ester of a primary alcohol.

4. The distribution of the "chloroesterase" in different tissues of normal dogs was observed by incubating equal amounts of dried, ether-extracted, aqueous tissue extracts with dichlorohydrin. The numbers in the following list represent the relative amounts of acidity produced with the respective tissues: liver 120, fundus (stomach) 100, pancreas 63, duodenum 31, ileum 19, pylorus (stomach) 17, muscle (skeletal) 15, brain 7, blood 6, heart 6. The relatively high activity in liver and pancreas tissues may be correlated with the active glandular character of these tissues, and the fact that they are relatively very active with respect to other common enzymes, *e.g.* lipase and erepsin. With exception of these two tissues, the "chloroesterase" activity in fundic stomach tissue is distinctly greater than it is in any other tissue so far studied. There is none of this activity in commercial pepsin.

5. In no case has it been possible to demonstrate a production of chloride ion corresponding in amount with the acidity developed, although this has been looked for under a variety of conditions by different methods. The acidity production therefore does not seem to be due to the formation of hydrochloric acid, and its possible significance in the normal gastric hydrochloric acid mechanism is rendered very questionable.

DISCUSSION.

The fact that it has not been possible to demonstrate chloride production along with the acidity production makes it appear unlikely what this "chloroesterase" is concerned with the normal gastric hydrochloric acid mechanism. On the other hand, there are two outstanding facts which lend weight to the probable significance of the enzyme in this connection. The amount of activity is always greater in the fundic than in the pyloric portion of the stomach, and greater in gastric than it is in any other tissue except liver. The activity is certainly a function of the presence of the chloride ester, and more specifically, the amount of activity is a function of the chemical nature of the organic chloride. We have thus far been unable to develop a plausible theory for the mechanism of the action of the chloride ester, aside from hydrochloric acid production, and we believe this phase of the question merits further investigation. Possibly the failure to show chloride ion production lies in the methods which have been used.

STUDIES ON THE PURIFICATION OF PEPSIN.

By T. L. McMEEKIN and F. C. KOCH.

(*From the Department of Physiological Chemistry, University of Chicago, Chicago.*)

Two quantitative methods for pepsin were devised based upon the rate of solution of properly prepared coagulated egg white. This rate of solution was determined in parallel by means of micro Kjeldahl estimations and refractive index readings. Remarkably consistent results were obtained by both methods and both were very satisfactorily applied upon unknown standard solutions of pepsin containing 0.03 to 8.9 mg. of pepsin.

In the purification studies advantage was taken of the specific absorption of pepsin upon properly treated coagulated egg white. The optimum conditions for completeness and greatest quantity of absorption of pepsin were found to be at a pH of 3 at room temperature. The pepsin can again be recovered from the egg white combination by treatment with dilute $NaHCO_3$ solution at a pH of 7.2 but even here there is considerable destruction of pepsin. By means of a 0.2 per cent disodium hydrogen phosphate solution 90 to 96 per cent recovery was shown. The pepsin thus absorbed and released was at least eight times as active as the original 1:3000 U.S.P. preparation started with. It was further purified by precipitation by half saturation with ammonium sulfate at a definite pH and then dialyzed. The ammonium sulfate-free material was evaporated to dryness *in vacuo* over phosphorus pentoxide. The product obtained was 16.9 to 17.8 times as active as the 1:3000 U.S.P. product. It is protein in nature, but not of glycoprotein or nucleoprotein character.

PEPSIN AND THE AUTOLYTIC PROTEASE.

By HARRY BAERNSTEIN AND H. C. BRADLEY.

(From the Laboratory of Physiological Chemistry, University of Wisconsin, Madison.)

Pepsin and the primary protease of tissues are so similar as to be easily confused. The older literature on the distribution of pepsin may therefore be erroneous and in need of revision. Potentiometer control of H ion concentrations is often impracticable in field experiments. By selecting acids of lower dissociation constants than HCl it is possible to set up digests whose pH values may be predicted with sufficient accuracy to permit the complete inhibition of the tissue protease while allowing pepsin to function catalytically. Fairly strong oxalic acid solutions are adapted to this purpose and by their use we are reinvestigating the distribution of pepsin. By means of it the stomachs of the herbivorous carp and the carnivorous cod are found devoid of pepsin, while the stomachs of the dogfish and mackerel shark are found to contain it.

A MODIFIED METHOD FOR THE DETERMINATION OF HIPPURIC ACID.

By WENDELL H. GRIFFITH.

(From the Department of Biological Chemistry, St. Louis University School of Medicine, St. Louis.)

Experimental studies on the synthesis of hippuric acid in the animal organism frequently necessitate the determination of hippuric acid in urines which may contain free benzoic acid and benzoylglycuronic acid in addition to hippuric acid. The methods of Folin and Flanders and of Kingsbury and Swanson cannot be used for the determination of hippuric acid in such urines because these procedures determine total benzoic acid rather than that fraction present as benzoylglycine. The methods of Dakin and of Snapper and Laqueur, which may be used for the determination of hippuric acid, are long and tedious.

The following procedure has been found to be more satisfactory than any of the above methods. 10 cc. of protein-free urine, acidified with HCl, are extracted with ether for 1 hour in a continuous extraction apparatus. The only nitrogenous substances present in the ether residue in significant amount are hippuric acid and urea. Complete decomposition of the urea is effected by shaking the residue with bromine water and then with NaOBr solution. Hippuric acid nitrogen is determined by the Kjeldahl method. Although hippuric acid is not readily soluble in ether, as much as 250 mg. can be extracted from 10 cc. of urine in 1 hour. The entire determination is carried out in the same 500 cc. Kjeldahl flask. This procedure gives accurate results and greatly reduces the number of laboratory manipulations which are necessary in the methods of Dakin and of Snapper and Laqueur.

THE ESTIMATION OF THE TOTAL PHOSPHORUS AND LIPOID PHOSPHORUS OF THE BLOOD.

By JOSEPH H. ROE.

(From the Department of Biochemistry, George Washington Medical School, Washington.)

The molybdic oxide colorimetric method introduced by Taylor and Miller, modified by Bell and Doisy, Briggs, and Benedict and

Theis is accurate when: (1) the blood is ashed at temperatures below 200°C.; (2) coloration is produced in acid concentrations ranging from 0.9 N to 1.9 N H_2SO_4; (3) ½ to 1 cc. of 5 per cent ammonium molybdate and 1 cc. of 1 per cent hydroquinone in 20 per cent sodium sulfite are used; (4) the phosphomolybdic acid mixture is boiled on a water bath for 15 minutes.

A NEW METHOD FOR POTASSIUM.

By CYRUS H. FISKE and GEORGES LITARCZEK.

(From the Department of Biological Chemistry, Harvard Medical School, Boston.)

The shortcomings of the cobalti-nitrite method, especially for the accurate analysis of urine, have led us to search for some other precipitating agent suitable for use with comparatively small quantities of potassium. For the analysis of potassium salt solutions, in the presence of a limited amount of sodium, the acid tartrate has much to recommend it. It can be precipitated quantitatively by means of alcoholic solutions of tartaric acid, and is susceptible to accurate titration with standard alkali. The tartrate method is, however, not specific. We have sought, therefore, to combine it in some way with the cobalti-nitrite precipitation, for the latter is an ideal means of isolating the potassium in fairly concentrated form, free from most other constituents of urine, blood, and other biological products.

The disadvantage of using a double precipitation method is more than offset by the fact that ashing of the urine, etc., is dispensed with. The same result is attained, with considerably less trouble, by ashing the cobalti-nitrite precipitate itself—it need not even be removed from the centrifuge tube in which it is thrown down. Decomposing the cobalti-nitrite with hot acid, as a preliminary to precipitation as the acid tartrate, would not do; the solution would contain ammonium salts and possibly organic matter, and cobalt (in solution) also interferes. By ashing, every interfering substance is either destroyed or driven off. The small amount of sodium which the precipitate contains is not thrown down by tartaric acid; and the cobalt is converted to the oxide, which is so little soluble that its presence does no harm, even if it is not removed at any time.

Ignition of the cobalti-nitrite precipitate leaves a residue containing the potassium in the form of nitrite, mixed with cobaltous oxide and some sodium nitrite. From this mixture, taken up in a small volume of water, alcoholic tartaric acid precipitates only the potassium, and the separation is complete in a few minutes. The acid potassium tartrate is then filtered off, washed with alcohol, and titrated with 0.02 N sodium hydroxide.

With this method, from 0.4 to 4 mg. of potassium may be determined within about 0.01 mg.

THE PHYSIOLOGICAL SIGNIFICANCE OF BACTERIAL DEAMINATION.

By HORACE B. SPEAKMAN.

(From the Department of Zymology, University of Toronto, Toronto, Canada.)

The process of deamination has been studied during a normal fermentation of carbohydrate by a bacillus. By chemical methods the products have been identified. The course of deamination has been correlated with vegetative growth and the primary and secondary oxidation of glucose.

Deamination is not associated with growth but with vigorous intercellular oxidation. The acid products are secreted by the cells, but the ammonia is utilized. Single amino acids and ammonium phosphate do not support growth, but they catalyze carbohydrate oxidation by resting cells. The specific dynamic action of amino acids is due to the catalytic effect of the liberated ammonia which passes through some unknown cycle within the cells.

STUDIES ON CHOLESTEROL.

THE RELATION OF THE SUPRARENALS AND THE SPLEEN TO CHOLESTEROL METABOLISM.

By F. S. RANDLES and ARTHUR KNUDSON.

(From the Department of Biological Chemistry, Albany Medical College, Albany.)

It has been held that the suprarenal gland is in some way closely associated with the cholesterol metabolism, and that this gland is capable of synthesizing cholesterol. In order to throw some light upon this question the adrenals were removed from about thirty rats. Most of these rats were placed on a cholesterol-free diet while the remainder were placed on the same diet with cholesterol added. After varying lengths of time the rats were killed and the cholesterol content of several tissues, particularly the blood, was studied. No marked departure from the normal cholesterol content could be noted in either series of rats.

The same experiment was tried, removing the spleens from rats, since this organ has been suggested as having a cholesterogenic function. In a series of twelve splenectomized rats, part on a cholesterol-free diet and part receiving cholesterol, no pronounced variation from the normal could be found in the cholesterol content of the blood.

THE VARIATIONS IN THE SUGAR CONTENT OF HUMAN URINE.

By FRANCIS B. KINGSBURY.

(From the Biochemical Laboratories of the Metropolitan Life Insurance Company, New York.)

53,599 urine specimens from applicants for life insurance and 12,239 specimens from employees of the company have been analyzed for sugar by the Benedict picric acid-acetone method modified for use in test-tubes. This method checked by the Folin-Berglund method gives values that average about 20 per cent higher than by the Folin-Berglund method with urines containing from 0.1 to 0.2 per cent sugar, but with urines containing from 0.2 to 0.5 per cent the average values in a series are the same by both methods.

Specimens from applicants for life insurance are sent to the home office from the whole territory east of the Rocky Mountains either because of abnormal findings by the examiners or because the medical division desires additional information. A certain number are sent in because the amount of insurance applied for is large, but since the rest are sent to the home office laboratory because there is doubt as to whether the applicant is a good risk the group as a whole is slightly less normal than the employee group with which they are compared. In this latter group the greater number have passed a physical examination on entering the employ of the company and the specimens obtained at this time and later at yearly intervals are the ones considered. The selection is not complete, however, because there are included the records of some who failed to pass the physical examination. The larger part of the employee group are young women.

A survey of the data shows that with both groups there is a very distinct line of demarcation at the 0.2 per cent level of sugar, and that there is very little variation in either group month by month of the total percentages of specimens showing less than 0.2 per cent of sugar. The average percentage of applicants whose specimens contained less than 0.2 per cent of sugar is 91.5 and for the employees 96.5. From these facts it seems probable that what we call the physiological sugar must in nearly all cases be an amount less than 0.2 per cent. At levels above 0.2 per cent there are relatively more of the applicant group than of the employee group for the same sugar level. This is particularly noticeable as the diabetic level is approached, and is probably due in part at least to attempts on the part of the applicants with known diabetes to obtain insurance.

SOME FACTORS INFLUENCING THE BASAL METABOLISM OF CHILDREN.

By GRACE MacLEOD and MARY SWARTZ ROSE.

(*From the Department of Nutrition, Teachers College, Columbia University, New York.*)

Basal metabolism determinations have been made on a group of 13 children (6 girls and 7 boys) living in an institution in the country and on another group of 10 children (6 girls and 4 boys) living in a city orphanage, first early in December and then again in late March or early April. The Benedict Student Apparatus was used, being checked against the Benedict Portable Respiration Apparatus both before and after it had been removed from the college laboratory to the orphanages.

It was found that the basal metabolism of the city children without exception was lower in April than in December by amounts ranging from 3.1 to 16.9 per cent. Of the country children 6 gave lower values in March than in December, the differences ranging from 1.0 to 7.5 per cent; 4 had higher basal rates in March than in December, by amounts ranging from 2.0 to 8.1 per cent; and 1 gave the same result in March as in December. In the total of 17 comparisons the December figure was greater than the

March figure in 12 cases, less than it in 4 cases, and equal to it in 1 case. It was known that the city children were housed more during the winter months than the country children. Both groups of children lead very active outdoor lives during the summer months, the city children being sent to camps in the country for the whole summer. We seem to be dealing here with an effect like that observed by both Lusk and Rapport in their dogs when they were brought under laboratory conditions after a vacation period in the country. Another large group of city children is being studied this winter with the object of further investigating this effect.

In the country institution it was also possible to compare the basal metabolism figure obtained on a child before rising out of bed in the morning with that obtained on the same child when he or she had slept in another building and had risen, washed, dressed, walked to the building where the apparatus was located, and had a rest period of 25 to 30 minutes before the determination was made. 18 comparisons were obtained on 12 different children. In 7 cases the figure when the determination was made before the child had risen was less than when he or she had come in from another building, in 8 cases it was greater, and in 3 cases it was the same. The differences were well within the range of individual variations from day to day, from 1.1 to 6.8 per cent. The conclusion would seem to be that it is immaterial whether the determination of the basal metabolism of a child is made before rising, in the bed where he has spent the night, or after a rest period following the child's arrival at the laboratory in the morning after having spent the night in his own home. This is in agreement with the results of the study of college women made by Benedict and Crofts at about the same time that this work on children was being done.

THE INFLUENCE OF PROLONGED ADMINISTRATION OF EGG UPON THE HEMOGLOBIN CONTENT OF CHILDREN'S BLOOD.

By MARY SWARTZ ROSE.

(From the Department of Nutrition and the Child Welfare Research Institute of Teachers College, Columbia University, New York.)

As part of an extended study of the place of the egg in children's diets, hemoglobin determinations and erythrocyte counts were made at intervals during 21 months (November, 1923, to July, 1925), on two groups of children between the ages of 2 and 6 years, the one group receiving a fairly good mixed diet from which eggs were excluded, the other being given in addition to a similar diet, one egg daily. The children were under constant supervision of a physician and a nutritionist, and the eggs were administered by the latter at the day nursery which all attended. The averages of the hemoglobin determinations on these children remaining in the group the whole time are as follows:

	Nov., 1923.	Mar., 1924.	July, 1924.	Nov., 1924.	Mar., 1925.	July, 1925.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Children receiving egg (10 cases).	82	83	89	79	86	84
" " no egg (9 cases).	88	84	77	72	80	80

The averages of the erythrocyte counts made in November, 1924, and July, 1925, on the whole number of children in each group at these times are as follows:

Children receiving egg (21 cases).		Difference.	Children receiving no egg (17 cases).		Difference.
Nov., 1924.	July, 1925.		Nov., 1924.	July, 1925.	
		per cent			*per cent*
4,300,348	4,290,900	−2.2	4,215,320	3,974,140	−5.7

There was a positive, though not large difference in percentage of hemoglobin and number of erythrocytes in favor of the children receiving egg, and there was a close parallelism between these blood findings and the general physical improvement of the children.

SPECIFIC DYNAMIC ACTION FROM THE STANDPOINT OF THE SECOND AND THIRD LAWS OF THERMODYNAMICS.

By ELLIOTT T. ADAMS.

(From the Department of Biochemistry, Harvard Medical School, Boston.)

The theory has been advanced by Oppenheimer that the cells of the body must do chemical work in order to bring about some of the metabolic transformations which follow the ingestion of food. This accounts, in his opinion, for the stimulating effect of some food substances upon metabolism. The results of free energy calculations are presented herewith which confirm this conception.

According to Lusk the following change in state takes place in the animal organism when alanine is ingested.

$$2CH_3{\cdot}CHNH_2{\cdot}COOH + H_2O + CO_2 = C_6H_{12}O_6 + CON_2H_4$$

It is a consequence of the second law that if this change in state does not take place spontaneously, work will be required to make it take place. With the aid of the third law it is possible to show that approximately 60,000 small calories of work will be required to make the change in state take place ($\Delta F = + 60{,}000$ calories). In order to do this work fuel must be burned in the organism. It is reasonable to suppose that the process will not take place in a reversible manner. Hence the fuel burned may be much larger than the minimum amount required to furnish the necessary work. This will explain, at least in part, the specific dynamic action of alanine. From this point of view the specific dynamic action of alanine is analogous (perhaps only in part) to the recovery process in muscle.

Lusk wrote the following equation to represent the metabolism of glycine.

$$3NH_2CH_2COOH + \tfrac{3}{2}CO_2 + \tfrac{3}{2}H_2O = C_6H_{12}O_6 + \tfrac{3}{2}CON_2H_4 + \tfrac{3}{2}O_2$$

The free energy increase for this change in state has been calculated with the aid of the third law and found to be approximately +196,000 calories. It is interesting to note that per mol of glucose formed the free energy increase is greater for the metabolism of glycine than for alanine. If Lusk's equation is physiologically correct this explains the relatively greater specific dynamic action of the former.

The calculations were made upon the assumption that the changes in state took place at 25°C. but otherwise under approximately the same conditions as are found in phlorhizinized dogs.

DEPOSIT PROTEIN: THE EFFECT OF THYROXIN ON THE DEPOSIT PROTEIN AFTER REDUCTION OF THE NITROGEN EXCRETION TO A MINIMAL LEVEL BY A PROLONGED PROTEIN-FREE DIET.

By HARRY J. DEUEL, Jr., KATHLEEN SANDIFORD, IRENE SANDIFORD, and WALTER M. BOOTHBY.

(From the Section of Clinical Metabolism, Mayo Foundation, Rochester, Minnesota.)

That thyroxin administration causes an increased protein catabolism as evidenced by the urinary nitrogen excretion is well known[2] but whether such an effect is indicative of a toxic action on protoplasmic tissue or the result of an increased rate of destruction of deposit protein is undecided. In the present experiment an attempt was made to remove a considerable proportion of the deposit protein, at least the more labile fraction, and then to determine what effect thyroxin exerts on protein metabolism. Therefore one of us (H. J. D.) went on a practically protein-free diet of about 1800 calories consisting of starch, sugar, centrifuged orange juice, lettuce, and definite amounts of salt solutions of Ca, Mg, Fe, Na, K, and Cl. This protein-free diet was continued for 54 days in which the carbohydrate at first was largely cooked cornstarch puddings and sugar; later, largely sugar, and finally cooked dried starch. A period of 8 days followed in which protein was taken in small amounts, and afterwards a second period of 9 days in which the protein-free diet was resumed. Urine specimens were collected in 24 hour samples. Determinations of the basal metabolism by the usual technique of Boothby and Sandiford were carried out daily.

The following determinations were carried out on the urine: volume, specific gravity, titratable acidity, total N, urea, ammonia, creatinine, creatine, uric acid, amino acid, total S, total sulfates inorganic sulfates, neutral and ethereal S, inorganic and total phosphates, Folin sugar, fermentable sugar, and Mg, Ca, K, Na, and Cl. Blood samples were obtained at frequent intervals and determinations of blood sugar, non-protein N, urea N, creatinine N, amino acid N, and uric acid N, and Cl (the latter both on plasma and whole blood) showed these constituents normal both at the beginning of the experiment, later when the protein metabolism had fallen to a very low level, and after the administration of thyroxin.

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[2] Boothby, W. M., Sandiford, I., Sandiford, K., and Slosse, J., *Ergebn. Physiol.*, 1925, xxiv, 728.

On the protein-free diet all the urinary constituents studied remained constant except that there was a marked decrease in urea nitrogen with a parallel decrease in total N while the S and P tended to follow the N excretion. When the N metabolism was lowered approximately to a minimal level by the protein-free diet, thyroxin was administered which resulted in a temporary increase of the N excretion, the maximum being reached on the 9th day. At this point thyroxin was administered to keep the elevation of the basal metabolic rate constant and the N excretion fell almost to the original level. That thyroxin causes no toxic breakdown of protoplasm is further shown by the constancy of the creatinine excretion and the absence of any significant changes in any of the nitrogenous or inorganic constituents of the urine except that of urea. The essential points of Folin's theory of protein metabolism are confirmed. Likewise the earlier investigations of Boothby, Sandiford, Sandiford, and Slosse,[2] which show that a shift upward in the thyroxin concentration causes an increase in the breakdown of deposit protein which returns to normal when the organism is adjusted to the change, are confirmed.

THE PERCENTAGE VARIATION OF THE NITROGEN PARTITION PRODUCTS IN THE URINE AS THE RESULT OF A PROLONGED PROTEIN-FREE DIET, TOGETHER WITH THE EFFECT THEREON OF THYROXIN AND SUBSEQUENT PROTEIN FEEDING.

By IRENE SANDIFORD, KATHLEEN SANDIFORD, HARRY J. DEUEL, Jr., and WALTER M. BOOTHBY.

(*From the Section of Clinical Metabolism, Mayo Foundation, Rochester, Minnesota.*)

As pointed out by Folin the fluctuations of the total N are produced almost entirely by changes in the urea N fraction and it is the only nitrogenous substance which suffers a relative as well as an absolute diminution with a decrease in the total protein metabolism. In our experiment (see previous abstract) at the lowest level of N elimination, 1.75 gm., the urea N was only 43 per cent of the total.

The absolute values of the total creatinine, uric acid, and amino acid N showed no significant fluctuations throughout the experiment but when expressed in percentages of the total N they are markedly increased as the total N decreases. The percentage of creatinine N rose to the height of 33 per cent of the total N on the day of lowest N elimination and was nearly equal to the percentage of urea N. The parallelism existing between the curves representing the percentages of creatinine, uric acid, and amino acid N is very striking and supports Folin's contention that these three substances indicate true endogenous protein metabolism. With the exception of the early part of the experiment when there is a decrease in the amount of ammonia eliminated as the total N decreases, the ammonia N remains constant in absolute amount and the percentage curve is similar to that for creatinine. Since the ammonia is concerned with maintaining the equilibrium between the excretion of acids and bases, this would indicate that no disturbance to this mechanism occurred as a result of the protein-free diet or the subsequent thyroxin administration. The rest N varied between 0.2 and 0.6 gm. and is indicative of the accuracy of the analytical data. The only significant fact that can be deduced from this is that thyroxin with an increased metabolism of stored protein does not cause the formation of any larger amount of any unknown nitrogenous waste product than occurs after the ingestion of ordinary protein food.

Calcium and magnesium show very slight fluctuations and none of any considerable magnitude; there are slight variations in the potassium which in general follow the more marked variations of sodium. The sum of all bases expressed in cc. of normal solution shows daily fluctuations of considerable magnitude which are caused in large part by changes in the elimination of sodium. However, the curve representing the sum of the acids (excluding the amino acids) almost exactly mirrors that of the bases.

THE EFFECT OF EXERCISE ON BREATHING IN EXPERIMENTAL ALKALOSIS PRODUCED BY INGESTED SODIUM BICARBONATE.

By ETHEL RONZONI.

(From the Laboratories of Biological Chemistry and Internal Medicine, Washington University, St. Louis.)

The average of three control experiments on three different individuals shows an accumulation of lactic acid during a period of from 3 to 5 minutes strenuous exercise, stair climbing, sufficient to raise the blood lactic acid from 22 mg. to 125 mg. per 100 cc. of blood, producing a measured fall in pH of from 0.2 to 0.25 pH. The CO_2 tension at this time, 2 to 3 minutes after exercise, is somewhat below the normal. During this early period after exercise when the lactic acid of the blood and the H ion concentration are still increasing, the respiration volume which has during exercise increased ten times drops off suddenly to seven times the normal respired volume.

If before equal amounts of exercise, enough sodium bicarbonate (30 gm.) is ingested to raise the pH from an average normal level of 7.38 (four cases) to 7.68 the CO_2 tension is raised on an average of 9 mm. and the respiration is depressed about 10 per cent of its total volume.

The result of exercise is to cause an almost equal increase in rate and volume as that observed in the control experiments, in two cases the rate was slightly less and the volume greater, in one case the rate was increased more but the volume showed the same increase, and in the fourth case the increase in both volume and rate checked with the control experiments. In all cases the dyspneic condition of the subject as judged from the appearances and subjective symptoms was decidedly worse.

The change in pH due to an accumulation of a similar amount of lactic acid brought the pH back almost to its normal level of 7.38 but in no case below that level. The CO_2 tension was below normal and the oxygen saturation above normal in all cases but one.

With an alkaline pH and no oxygen-want it is difficult to explain the initiation of respiratory stimulation unless we assume the adjustment of the respiratory center to an alkaline pH, or some reflex mechanism; once initiated it may be kept up by the mechanism suggested by Gesell, that is, the increased activity of the respiratory cells increases the CO_2 tension or lactic acid within the center resulting in stimulation due to its inability to diffuse out. We also fall into difficulties when we try to explain the sudden drop in rate and volume at the end of exercise when the lactic acid and H ion concentration are still increasing and the CO_2 tension is also increasing. Fatigue of the center is a possible explanation but this seems unlikely when we consider the short period of exercise. The removal of some reflex stimulation seems to be the only possible explanation left.

CARBON DIOXIDE TENSION OF CEREBROSPINAL FLUID.

By A. T. SHOHL and S. KARELITZ.

(From the Department of Pediatrics, Yale University, School of Medicine, New Haven.)

By CO_2 absorption curves, spinal fluid was found not to differ from a corresponding concentration of $NaHCO_3$ in 0.9 per cent NaCl. The pK'_1 value thus determined was less accurate than that calculated from the recently published activity coefficient of Hastings and Sendroy according to the equations

$$pK'_1 = pK_1 - 0.5\sqrt{\ } = 6.13 \text{ and} \tag{1}$$

$$p_{aH} = pK'_1 + \log R \tag{2}$$

By the equation

$$H_2CO_3 = C\,\frac{1}{R+1} \tag{3}$$

the value of H_2CO_3 in spinal fluid is found to be higher than that of venous blood. The normal tension of CO_2 in cerebrospinal fluid is approximately 5 to 10 mm. higher than that calculated for blood with values of about 45 to 50 mm.

THE BIOCHEMICAL OXIDATION OF FATTY ACIDS.

By H. GREGG SMITH.

(From the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, University of Rochester, Rochester, New York.)

In 1909, Leathes expressed the theory that the body introduces ethylene linkages in the long chain fatty acids in order to form points of weakness at which these acids are first oxidized, forming two or more short chain acids which are subsequently oxidized in the ordinary manner, beta-oxidation. This idea has been generally accepted without question. The indirect evidence which is available does not support the theory, but is questionable and the present work is intended to obtain direct evidence for or against the theory. If the short chain mono- and dibasic acids, formed in the laboratory oxidation of the unsaturated acids, are fed to animals and are utilized, the theory stands; if they are not burned, the theory lacks direct support and falls. Azelaic acid, the most common of the dibasic acids produced from unsaturated acids, and pelargonic acid, produced in the oxidation of ordinary oleic acid, were fed to dogs. Pelargonic acid was completely burned, but 40 per cent of the azelaic acid was excreted, giving partial evidence that the Leathes theory lacks direct support.

LIPID EXCRETION BY DOGS ON A LIPID-FREE DIET.

By WARREN M. SPERRY.

(From the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, University of Rochester, Rochester, New York.)

Previously reported work has indicated that the lipids found in the feces of dogs on a normal diet consist of a basal excretion, represented by that occurring with lipid-free diets, together with a smaller amount of diet lipid which has either escaped absorption or has been absorbed and reexcreted. Because of the value which a thorough knowledge of this basal excretion might have in helping solve problems of lipid metabolism, it seemed desirable to subject it to a rather detailed examination. About 60 analyses of the feces of animals receiving no lipid in their food have failed to show a single case in which fatty substances were absent or nearly absent and, moreover, the composition of the material excreted by different animals has been found to be quite uniform throughout the work. In order to eliminate any possibility of error due to poor demarcation, experiments have been carried out over long periods of time and even after 5 weeks lipids continued to be excreted in nearly as large amount and of the same composition as at the beginning. Analysis has shown that the fatty acids, which make up about 60 per cent of the basal lipid excretion, are composed in the main of palmitic, stearic, and very probably oleic acids and it is, therefore, difficult to explain this as a real excretion of waste products; and for the same reason it seems hardly probable that these fecal lipids can reach the intestine by way of the bile, since, if they did, we would expect them to be reabsorbed. This view is supported by preliminary experiments with bile fistula dogs receiving strictly lipid-free diets. Lipids continued to be excreted by such animals in larger amounts than the same animals excreted while they were still normal.

SOME EFFECTS OF POSTERIOR PITUITARY ABLATION IN THE RAT.

BY G. L. FOSTER AND PHILIP E. SMITH.

(From the Departments of Biochemistry and Anatomy, University of California, Berkeley.)

An operation has been developed by one of us which makes it possible to ablate either the entire hypophysis, or only its posterior lobe (neural and intermediate lobe) component, leaving the capsule of the gland, the pituitary stalk, and the pars tuberalis intact. Any brain injury appears to be precluded. In the posterior lobe ablation the anterior pituitary is also usually partly removed, a sufficient amount being left, however, so that no anterior lobe deficiency is displayed by the animal. The operative approach leads directly to the nasal surface of the sphenoid between the medial pterygoid laminæ without opening the oral cavity.

In six animals the posterior lobe has been either completely or nearly completely ablated as shown by a study of serial sections of this region. Growth, the sex cycles, and the capacity to bear young appeared to be not impaired; nor, as shown by the autopsy, were there significant weight changes or structural alterations in the thyroids, adrenals, or ovaries.

Studies were made on these animals of the basal metabolism and the specific dynamic action of glycocoll injected intraperitoneally, using a closed circuit respiration apparatus adapted for short periods. The basal metabolism in calories per kilo per hour was found to be not different from that of the normal animal, but the specific dynamic action of glycocoll, while well marked in the normal rat, was entirely absent in the case of the operated animals. Daily injections of posterior lobe extract for several days brought about the normal response to amino acid injection in the animals which had undergone posterior lobe ablation. Cessation of the injections for several days resulted in the disappearance of the amino acid stimulation, which was again restored by a renewal of the posterior lobe injections.

THE EFFECT OF COMPENSATORY HYPERTROPHY ON THE FAT, CHOLESTEROL, AND PHOSPHATIDE CONTENT OF THE SUPRARENAL GLAND.

By EMIL J. BAUMANN.

(*From the Chemistry Laboratory, Montefiore Hospital, New York.*)

The suprarenal glands of rabbits were removed under ether anesthesia in two stages. The interval between removal of right and left glands varied between 7 and 73 days. The glands were analyzed for their fat (total ether-alcohol extract soluble in chloroform minus cholesterol and phosphatides), cholesterol, and phosphatide contents.

In normal animals the left suprarenal gland is almost always larger than the right (about 10 to 20 per cent). The per cents of cholesterol and phosphatides are greater in the smaller (right) glands so that the total amount of cholesterol and phosphatides is about the same in both glands.

Compensatory hypertrophy of the left suprarenal usually results in an increase in the per cent of phosphatides and consequently in the total amount also. After complete compensation the per cent of phosphatides seems to decrease although the total amount is somewhat higher.

Considerable variation in the cholesterol content of suprarenal glands of different animals was noted. The differences in the composition of right and left suprarenals of rabbits in the hypertrophy experiments were of the same magnitude as the variations in normal right and left suprarenals. If cholesterol were a *specific* element in suprarenal gland function, as has been maintained so often, we should then expect an unequivocal decrease in the per cent of cholesterol during the active stage of hypertrophy of the gland. This we were unable to show.

THE INTERMEDIARY METABOLISM OF THE AROMATIC AMINO ACIDS.

PHENYLALANINE.

By NOEL F. SHAMBAUGH* and HOWARD B. LEWIS.

(*From the Laboratory of Physiological Chemistry, University of Michigan, Ann Arbor.*)

Phenylalanine has been administered *per os* and subcutaneously to rabbits in doses of approximately 1.0 gm. per kilo and the changes in the blood and urine have been studied. 3 hours after the administration of the phenylalanine, the phenol content of the blood as determined by Benedict's *p*-nitroaniline method had increased to about twice the normal value. The total phenols of the urine (Folin-Denis) increased indicating that about 10 per cent of the aromatic nucleus of the phenylalanine had been eliminated in the urine as phenols. There was no corresponding increase in urinary amino acid nitrogen. The formation of the phenols was not due to intestinal microorganisms since the excretion of phenols was as great after subcutaneous injection of phenylalanine as after oral administration. The increased phenol content was due in part to the presence of an *o*-diphenol derivative which gave the typical reaction of *o*-diphenols with ferric chloride. The substance which gave the reaction has been extracted from the urine and did not contain amino acid nitrogen. After the administration of tyrosine, the phenol content of blood and urine increased, but no evidence of the presence of an *o*-diphenol was obtained. The results are considered to indicate that in the oxidation of the aromatic group of phenylalanine, a diphenol is formed as an intermediary product, and that tyrosine and phenylalanine do not follow the same paths in intermediary metabolism in the organism of the rabbit.

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* Medical Fellow of the National Research Council in Physiological Chemistry.

THE EFFECT OF ORANGE JUICE ON THE CALCIUM, PHOSPHORUS, MAGNESIUM, AND NITROGEN RETENTION AND URINARY ORGANIC ACIDS OF GROWING CHILDREN.

By MARGARET S. CHANEY AND KATHARINE BLUNT.

(*From the Department of Home Economics, University of Chicago, Chicago.*)

A study was conducted on two growing girls, 10 and 11 years old, to determine the effect of orange juice on calcium, phosphorus, magnesium, and nitrogen metabolism.

The basal diet consisting of oatmeal, rice, flour, bread, potato, sugar, oleomargarine fat, lean beef, and whole milk powder was ingested for a preliminary adjustment period and a 3 day collec-
period. Then orange juice, 600 to 700 cc. daily, was added to
t; another adjustment period and collection period followed.
n interval of 2½ months the experiment was again performed
same girls.

Calcium assimilation was decidedly benefited when oranges formed a part of the diet. The increased retention was considerably greater than the calcium added in the oranges and greater than might be expected from a stimulus to retention caused by a larger calcium intake.

The increase in phosphorus retention was even more marked than that of calcium, more than three times as much phosphorus both in gm. and percentage of intake, being assimilated by the body when orange juice was added to the diet.

The magnesium retention was also increased, although to a less marked extent than that of calcium and phosphorus.

Nitrogen assimilation was greater when orange juice was ingested, even though the nitrogen intake was not altered.

Urinary ammonia was decreased, and urinary pH and organic acids increased, thus confirming the work of Blatherwick and Long. The increase of organic acids amounted to approximately 7 per cent of the citric acid of the orange juice ingested.

A marked increase in the children's weight was observed.

CALCIUM, PHOSPHORUS, AND MAGNESIUM METABOLISM IN THE RAT.

By GRACE MEDES.

(*From the Department of Physiological Chemistry, University of Minnesota Medical School, Minneapolis.*)

Pedigreed rats 32 to 48 days old were fed for varying periods on weighed portions of diets of casein, starch, sugar, vitamins A (extracted from spinach) and B (Harris yeast vitamin) and salt mixtures in which the ratios of P to Ca, P to Mg, and Ca to Mg were varied in the different experiments.

At the close of each experiment the rats were ashed and the Ca, Mg, and P determined. The excreta and samples of the food were also ashed and the same elements determined and from these data the composition of rats at the beginning of the experiments was estimated.

Lantern slide tables were presented giving the metabolic balance in Ca, Mg, and P showing the effect of high and low dietary Ca, Mg, and P on the metabolic balance of these elements.

THE PHOSPHORUS CONTENT OF THE BODY IN RELATION TO AGE, GROWTH, AND FOOD.

By H. C. SHERMAN and E. J. QUINN.

(From the Department of Chemistry, Columbia University, New York.)

The phosphorus content of the body of the normal white rat is about 0.34 per cent at birth and increases during growth and development, at first rapidly and then more gradually, to from 0.60 to 0.78 per cent in the full grown animal. Thus the body during growth multiplies its birth phosphorus about twice as many fold as its birth weight. The gain of phosphorus by the body has been studied in detail with reference to its relations to the age and general growth, the calcification of the bones as indicated by increase of body calcium, the breeding records of the females, and the influence both of dietary deficiencies and of changes in the character of the food within normal limits.

STUDIES ON INSULIN.

By N. R. BLATHERWICK, L. C. MAXWELL, JOHN BERGER, and MELVILLE SAHYUN.

(From the Chemical Laboratory of the Potter Metabolic Clinic, Santa Barbara Cottage Hospital, Santa Barbara.)

A Simpler Method of Preparing Purer Insulin.—In this method, the procedure of Scott and Best[3] is followed up to the point where they precipitate with $(NH_4)_2SO_4$. In place of the ammonium salt we saturate by adding 22 gm. of NaCl per 100 cc. The salt precipitate is dissolved in the original volume of water and again saturated with NaCl without changing the reaction. This moist precipitate is dissolved in sufficient 95 per cent alcohol to make a concentration of 75 per cent, filtered or centrifuged to remove insoluble matter, and the insulin is again precipitated by adding to two volumes of ether. The ether precipitate is dissolved in water and brought to the isoelectric point with NaOH. After the third isoelectric precipitation from water, a yield of about 1000 clinical units per kilo of pancreas is obtained. The nitrogen amounts to approximately 0.005 mg. per unit. This insulin precipitates at a pH of about 5. The second saturation with NaCl appears to remove most of the contaminating pH 7 protein. Further saltings do not effect a greater purification.

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[3] Scott, D. A., and Best, C. H., *Ind. and Eng. Chem.*, 1925, xvii, 238.

Purification of Insulin by Fractional Precipitation with Alcohol.—A less pure insulin is obtained when but one salt precipitation is made in the above method. This crude insulin, when dissolved in alcohol, precipitated with ether, and precipitated once at the isoelectric point from water, contains at least two substances. These can be rather completely separated utilizing their different solubilities in alcohol. If the isoelectric precipitate is dissolved in a minimum amount of water and NaOH, alcohol added to a concentration of about 75 per cent (hydrometer), and HCl added to a pH of about 6.6, a dirty, meaty-looking precipitate is formed. This material is relatively impure. It carries most of the red-brown color of the original solution. Upon the addition of absolute alcohol to the supernatant fluid from this precipitate in amount to make a concentration of about 92 per cent alcohol, and upon adjusting to a slightly more acid reaction, a flocculent, white precipitate forms. This material is much purer than the other fraction and carries most of the activity. The results of a typical experiment were: mg. N per unit in the first fraction 0.050, in the second 0.0051. These observations confirm and extend the findings of Somogyi, Doisy, and Shaffer.[4]

Dialysis.—The material used in these experiments was extracted according to Collip's method. After concentration, and removal of fat with ether, the aqueous solution was saturated with NaCl. This precipitate was dissolved in alcohol and precipitated by adding to ether. The aqueous solution of this precipitate was dialyzed. Before dialysis the solutions were brought just through the isoelectric point with NaOH. Rather thin collodion membranes were used. Dialysis was continued for about 5 hours with three changes of distilled water. The first experiment showed that 4 per cent of the active material dialyzed. 63 per cent of the potency was found in the isoelectric precipitate of the residue and 33 per cent remained in the filtrate. The biuret reaction of the concentrated dialysate was a distinct pink in contrast with the violet color of the isoelectric material. Under the experimental conditions a precipitate formed within the sac during dialysis. This precipitate was removed and kept separate from the isoelectric material obtained from the residual fluid. The sac precipitate was less pure and somewhat more difficult to purify than the one obtained by isoelectric precipitation. Repeated precipitations at the isoelectric point brought the N content of both fractions to less than 0.005 mg. per clinical unit. The isoelectric point of these preparations when purified was about pH 6 (colorimetric). Dialysis permits a sharper precipitation at the isoelectric point due to the low salt concentration. Loss of potency is therefore not so great.

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[4] Somogyi, M., Doisy, E. A., and Shaffer, P. A., *J. Biol. Chem.*, 1924, lx, 31.

Heating.—Fairly crude or purified insulin in aqueous solution is precipitable by heat. The necessary conditions are: (1) the pH should be 4.0 to 4.2, (2) the solution should contain from 50 to 100 units per cc., and (3) and NaCl content should be nearly 1 per cent. We heat at a temperature of 80°C. for about 1.5 hours. The precipitate which forms is usually colloidal and is not to be confused with an isoelectric precipitate. If conditions are right, the potency is practically regained in the heat precipitate and impurities remain in the filtrate.

THE RELATION OF THE PARATHYROID GLANDS TO THE CALCIUM OF THE BLOOD.

By ISIDOR GREENWALD.

(*From the Harriman Research Laboratory, The Roosevelt Hospital, New York.*)

Although, by the addition of sodium citrate, it is possible to prepare simple saline solutions that will keep calcium phosphate in solution in quantities comparable to those found in serum, the solubility of calcium phosphate in serum is not due to the presence of citrate. Serum does not contain the requisite amount of citrate. Solutions of serum proteins, or of crystallized egg albumin, to which calcium chloride and sodium phosphate have been added furnish solutions containing calcium phosphate in quantities greater than those normally present in serum. However, it has also been found possible to prepare concentrated dialysates from serum that are protein-free yet contain, at pH ranging from 7.2 to 8.5, quantities of calcium phosphate far in excess of those possible in simple saline solutions. When such solutions are acidified, boiled for 1 hour, cooled, and adjusted to original volume and reaction, most of the calcium is precipitated and the filtrates contain only small amounts. It is suggested that it may be through the production of increased amounts of this diffusible, thermolabile substance that the parathyroid hormone operates to increase the calcium concentration of the serum and that it is because of the absence of this substance that the concentration of calcium in the serum of parathyroidectomized dogs decreases.

THE PARATHYROID HORMONE.

By AXEL M. HJORT, H. B. NORTH, and F. H. TENDICK.

(From the Department of Chemical Research, Parke, Davis and Company, Detroit.)

A study was made of the uniformity and constancy in action of extracts prepared identically from one lot of acetone-desiccated external bovine parathyroid glands. Ten normal dogs, both male and female (10 to 12 kilos), were employed. The experiments were performed at 2 week intervals to avoid possible complication of induced anemia. Two females passed through normal gestation periods during the course of the experiments. The effect of calcium-rich diets upon the response of the animals to the extracts was also studied. In every case save one, the extracts were given subcutaneously, the excepted case intravenously. One experiment was performed with each of two other groups of normal dogs to study the diurnal variations of the serum calcium, and the relationship between dosage and body weight.

Barring extremely young and old dogs no direct relationship between induced hypercalcemia and age, sex, weight, pregnancy, and diet was observed. Different dogs varied as much in their response to the same dosage of extract as the same dog at different times. In some cases, a very slight early transient rise in serum calcium followed intravenous administration of the extract; in others, no increase was induced.

The diurnal variation of serum calcium in fasting normal dogs may be as much as 10 per cent.

THE CONTROL OF THE MUSCULAR RIGIDITY OF CATATONIC PRÆCOX PATIENTS BY PARATHYROID HORMONE.

By JOSEPH M. LOONEY.

(From the Biochemical Laboratory of the Sheppard and Enoch Pratt Hospital, Towson, Maryland.)

In previous communications[5,6] from this laboratory it was shown that the creatine content of the blood varied with the muscular tension of the individual. In catatonic præcox patients showing marked rigidity there is an increase in creatine measured in per cent of non-protein nitrogen from 8.4 to 12.9. Various investigators have pointed out that there is an increase in guanidine excretion in cases of tetany and that there is also a decrease in the calcium content of the blood. Because of the close chemical relation between methyl guanidine and creatine it was felt that there might also be some change in the calcium content of the blood of these markedly catatonic cases.

In a series of eleven cases showing various degrees of rigidity a slight decrease in the calcium content of the blood was obtained. The average content was 9.1 mg. per 100 cc. The calcium content of the serum was increased from 0.4 mg. to 14.0 mg. by injections of parathyroid hormone (Collip). This figure was maintained for several days and there was a decrease in the muscular rigidity so that, whereas before the injections it was impossible to open the patient's hands, afterwards almost complete extension was obtained.

25 units of parathyroid hormone can be injected every 8 hours over a period of 5 days without danger.

I wish to express my appreciation to Dr. Collip and to the Eli Lilly Company for their courtesy in supplying me with the parathyroid hormone.

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[5] Looney, J. M., *Am. J. Physiol.*, 1924, lxix, 639.

[6] Looney, J. M., *Am. J. Psychol.*, 1924, lv, 29.

PLASMA PROTEINS IN EXPERIMENTAL ANHYDREMIA.

By MEYER BODANSKY.

(From the Laboratory of Biological Chemistry, University of Texas School of Medicine, Galveston, and the Department of Chemistry, Stanford University, California.)

A study has been made of the plasma proteins in a variety of conditions associated with concentration of the blood. Following the administration of symmetrical diisopropyl hydrazine hydrochloride, the per cent decrease of the plasma volume may exceed 30, and is associated with an increased concentration of the plasma proteins which does not quite parallel the plasma volume decrease. Thus, in two dogs, decreases in plasma volume of 30 and 34 per cent were observed, whereas the protein concentrations increased but 25 and 31 per cent respectively, showing that a small amount of protein probably escaped from the blood to the tissues.

Similar but less marked changes were likewise observed in intoxications with hydrazine and 2-2′-azobispropane.

In pilocarpine anhydremia, concentration of the blood and of the plasma proteins may run parallel. In one case, however, in which hemorrhagic extravasation in the liver and kidneys was especially severe, the protein increase was only 4.0 per cent as compared with a 13.0 per cent decrease, from the normal, of the plasma.

On the other hand, in histamine shock, both the plasma volume and protein content diminish. Thus with plasma volume decreases of 38.0 and 14.0 per cent, protein decreases of 8.6 and 16.0 per cent respectively were observed, showing that in histamine shock the permeability of the capillary wall for proteins is much greater than in the other forms of anhydremia studied.

The albumin-globulin ratio of the blood decreased in all the forms of anhydremia studied with the exception of two cases of histamine shock where no significant variations from the normal were observed. An attempt was made to determine variations from the normal of the various globulin and albumin fractions by Howe's methods. No constant changes were observed in the anhydremic state except for fairly consistent increases of the fibrinogen and euglobulin fractions.

Except in hydrazine poisoning, concentration of the blood was always accompanied by a decrease in the carbon dioxide tension.

THE USE OF THE HOPKINS-COLE GLYOXYLIC ACID REAGENT FOR THE QUANTITATIVE DETERMINATION OF FREE TRYPTOPHANE IN BLOOD.

By C. A. CARY.

(*From the Research Laboratories of the Bureau of Dairying, United States Department of Agriculture, Beltsville, Maryland.*)

To determine the free tryptophane in the blood or the blood plasma of cows, the protein was removed as in the amino N procedure used in this laboratory[7,8] except that the trichloroacetic acid was omitted. The concentrations were done *in vacuo.* The aliquots, after the second kaolin treatment, were brought to about twice the volumes of the blood or plasma represented in them and precipitated with H_2SO_4 (7 per cent by volume) and $HgSO_4$ (10 per cent) by allowing to stand in ice box overnight. These precipitates were then filtered onto asbestos, washed with 5 per cent H_2SO_4 and returned to the original vessels along with the asbestos mats. 20 cc. of freshly prepared glyoxylic acid reagent[9] and 4 drops of a solution of 25 per cent mercuric sulfate in 10 per cent H_2SO_4 were added to each. After 48 hours at room temperature these mixtures were filtered and compared with tryptophane standards similarly precipitated and treated. The color, recovery of added tryptophane, and agreement in duplicate determinations were very satisfactory. Ths use of CCl_3COOH or other protein precipitants in addition to the above procedure did not alter the results. A portion of the color (15 to 20 per cent) may be due to indole derivatives other than tryptophane in the blood or to decomposition products of tryptophane formed in the procedure. No correction for this is made in the data given in the paper following.

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[7] Cary, C. A., *J. Biol. Chem.*, 1920, xliii, 477.

[8] Cary, C. A., and Meigs, E. B., *J. Agric. Research*, 1924, xxix, 603.

[9] Reagent contains 20 cc. of H_2SO_4 solution (21 cc. of 95 per cent H_2SO_4 + 5 cc. H_2O) and 1 cc. of glyoxylic acid solution (100 cc. of saturated oxalic acid and 6 gm. of 5 per cent sodium amalgam).

THE FREE TRYPTOPHANE IN COW'S BLOOD AND ITS UTILIZATION BY THE MAMMARY GLAND.

By C. A. CARY.

(From the Research Laboratories of the Bureau of Dairying, United States Department of Agriculture, Beltsville, Maryland.)

In eleven samples of cow's blood the free tryptophane varied from 0.95 to 1.47 mg. per 100 cc.; in seven samples of plasma it varied from 0.71 to 1.35 mg. This tryptophane is taken out of the plasma of the blood and used by the mammary gland in the secretion of milk. This was shown by comparing the concentrations of it in plasma from samples of blood taken simultaneously from the jugular and mammary (abdominal subcutaneous) veins. In two experiments with milking cows the free tryptophane in the mammary blood plasma was 19 per cent and 17.7 per cent lower than in the jugular. With three cows that were not milking these differences were 0.7, 3.1, and 8.1 per cent, with a flow of blood through the glands that was only a fraction of that in the milking cows.

THE NORMAL URINARY PIGMENT.

By DAVID L. DRABKIN.*

(From the Laboratory of Physiological Chemistry, Yale University, New Haven.)

The normal pigment of urine, commonly termed *urochrome*, was studied under conditions in which the presence of extraneous pigments was eliminated from the diet and such substances as urobilin and hematoporphyrin were excluded. Most of the experiments were performed upon dogs, but the urines of rats and humans were also studied. The daily output of pigment was determined colorimetrically. The standard used was a mixture of dyestuffs which simulated closely the color and fluorescence of urine. The determinations were expressed empirically in units.

It was found that, under controlled conditions, the quantity of urinary pigment eliminated from day to day is constant. The output upon a practically colorless food mixture, used as the standard diet, was determined. A study of the effect of changes in the character of the diet upon the color of the urine indicated that the amount of urinary pigment is essentially independent of the diet. The administration of alkalies decreased the quantity of urine pigment. Marked increases in pigment output followed the administration of acids, fasting, and, above all, the augmented metabolism produced by thyroxin, adrenalin, and phlorhizin. In a severe case of Graves' disease the output of the urinary pigment from day to day was found to be a good index of the patient's metabolic rate and clinical progress. The volume of urine was found to vary, in a general way, with the amount of pigment, but diuresis *per se* caused no change in pigment output.

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* Medical Fellow of the National Research Council.

That the normal urinary pigment is an endogenous product is further suggested by the comparative study of several species. Calculated upon a kilogram basis, a rat eliminates in the urine 7.9 times the amount of pigment of an adult man, 3.6 times that of a medium sized dog, 2.7 times that of an infant, and 1.4 times that of a 2 months old puppy. Furthermore, the figures for the pigment output of the different species plotted against the surface area give a straight line.

FRACTIONAL PRECIPITATION OF PROTEIN IN URINE.

By ANTON R. ROSE and W. G. EXTRON.

(From the Laboratory of The Prudential Insurance Company of America, Newark.)

A procedure for fractional precipitation of protein in urine by ammonium sulfate has been devised, and applied to random proteinuria specimens, also to some controlled cases. The precipitates were measured by the skopometer, a turbidity meter with "disappearance criteria." Globulin occurs in most specimens of proteinuria ranging from 0 to 90 per cent of the total protein. In no specimen containing 100 mg. or more of protein per 100 cc. did the globulin constitute one-third of the total protein and globulin-free specimens occurred in both protein-rich and protein-poor samples. In some cases the globulin-albumin ratio was constant throughout the 24 hour day; in others it varied considerably.

HYPOGLYCEMIA WITH CONVULSIONS IN PHLORHIZIN DIABETES.

By M. WIERZUCHOWSKI.

(*From the Department of Physiology, Cornell University Medical College, New York City.*)

Ten dogs were fasted and were daily phlorhizinized until they became comatose. In three such animals convulsions began during the comatose state when the blood sugar level reached 0.06 per cent. Oral administration of glucose relieved the convulsions in 20 minutes. At the same time the ketone bodies disappeared from the breath and urine and also from the blood, as indicated by the rise in its carbon dioxide-combining power; coma disappeared completely, urinary nitrogen fell (Deuel and Chambers), and the dog reacted as does one in normal fasting. These beneficial phenomena were due to an increase in the blood sugar level, for as the blood sugar again decreased, ketone bodies reappeared in the breath and urine, the carbon dioxide-combining power of the blood fell, coma again intervened, and finally, when the blood sugar fell to a level somewhat below that observed in the first instance, convulsions took place as before. About 80 per cent of the glucose ingested was recovered as extra sugar in the urine. The two lethal syndromes, hypoglycemic convulsions and coma, were therefore completely relieved by glucose administration to phlorhizinized dogs. Whether the ingested glucose was oxidized or not will be shown in a subsequent report.

A CHEMICAL AND ELECTRONIC BASIS FOR THE THEORY OF ANTIKETOGENESIS.

By EDWARD S. WEST.

(*From the Department of Biological Chemistry, Washington University School of Medicine, St. Louis.*)

According to the modern theory of antiketogenesis in the animal body, acetoacetic acid or other ketogenic molecule is condensed with a sugar or sugar derivative to form a compound which has a higher reduction potential, or is more easily oxidized than the unchanged ketogenic molecule.

It has been found that methylene-, ethylidene-, benzylidene-, vanillylidene-, and furfurylidene-bis-acetoacetic ester (acid), and also ethyl α'-acetoacetic ester-α-methyl-β-furan carboxylate and a condensation product of glucose and acetoacetic ester are much more powerful reducing agents than unchanged acetoacetic ester (acid). In all of these cases the carbon atom of the acetoacetic acid chain which is linked to two mobile hydrogen atoms has been linked to carbon of the condensing molecule by one valence and still retains one mobile hydrogen atom. Condensation products of acetoacetic ester (acid) in which this carbon atom is linked to carbon of the condensing molecule by two valences and retains no mobile hydrogen show much less reducing power.

From a consideration of the electronic configuration of acetoacetic acid and its condensation products the latter should possess, as actually found, the higher reduction potentials.

The theory of antiketogenesis as put forward by Woodyatt, Shaffer, etc., therefore, has a firm basis of fact from the standpoint of the ease of oxidation of condensation products of acetoacetic ester (acid).

THE EFFECT OF KETOSIS ON INSULIN ACTION IN THE RABBIT.

By ELMER L. SEVRINGHAUS.

(*From the Department of Physiological Chemistry, University of Wisconsin, Madison.*)

The occurrence of ketosis due to carbohydrate deprivation in the healthy adult human produces a reduced rate of glucose utilization following a simple meal. This result is apparent following the first intake of carbohydrate, after which it disappears. To determine the nature of the responsible factors rabbits were injected with insulin and simultaneously with acetone or with sodium acetoacetate. Controls were injected with either the insulin or the ketone body. The rabbits were fasted for 24 hours before use. Controls and test animals were of nearly the same weight. The insulin dosage was 3 clinical units per 2 kilos. Blood sugar concentration was determined in venous blood from the ears by the Folin-Wu method or by Gibson's adaptation for 0.1 cc. samples. Acetone was prepared by redistillation. Sodium acetoacetate was prepared from the ethyl acetoacetate by saponification with alkali, preparation of the soluble barium salt, and decomposition with sodium sulfate to remove barium. The product was not toxic except in the effect described below.

The animals injected with acetone showed no more variation in blood sugar level than untreated animals. Insulin produced hypoglycemia in a typical manner. Acetone dosage was from 0.05 to 0.4 cc. per kilo. With the higher doses general depression of the animal was seen. The animals injected with sodium acetoacetate showed a tendency to hyperglycemia. When insulin was injected simultaneously there was a retardation in the decline of the blood sugar level and the degree of hypoglycemia was less marked than in control animals, with insulin alone. The effects of sodium acetoacetate on insulin action are not permanent. The effect of acetoacetate on insulin *in vitro*, and the results of injection of sodium beta-oxybutyrate are being sought. These results are believed to be important in disclosing the significance of insulin therapy in non-diabetic ketosis and the relative inefficiency of insulin in diabetic coma.

THE EFFECT OF GLUCOSE AND INSULIN INJECTION UPON ARTIFICIAL KETOSIS OF NORMAL AND DIABETIC DOGS.

By THEODORE E. FRIEDEMANN, M. SOMOGYI, and P. K. WEBB.

(*From the Department of Biological Chemistry, Washington University School of Medicine, St. Louis.*)

Freshly prepared solutions of sodium acetoacetate were injected intravenously into dogs at approximately constant rates for periods of 6 to 20 hours. Up to a rate of injection of about 2 mM per kilo per hour hardly perceptible amounts of acetoacetate were excreted.[10] Up to a rate of 4 or 5 mM about 90 per cent was burned, only 5 to 9 per cent being excreted in the urine, and less than 1 per cent exhaled as acetone. Injections of insulin and glucose did not increase the tolerance.[11]

Two depancreatized dogs showed a normal acetoacetate tolerance, in spite of typical D:N ratios. The injections of phlorhizin into two normal dogs during the course of the experiment failed to affect their acetoacetate tolerance. The tolerance was definitely lowered after prolonged fasting and phlorhizin treatment, being in one case thus reduced to 50 per cent and in another to 70 per cent of the normal. Injection of insulin and glucose into one of these animals resulted in normal tolerance after 3 hours.

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[10] Wilder, R. M., *J. Biol. Chem.*, 1917, xxxi, 59.

[11] Burn, J. H., *J. Physiol.*, 1925, lx, p. xvi.

Base was excreted as $NaHCO_3$ coincident with and paralleling the acetoacetate injection. The base excreted was almost equal to the amount of acetoacetate disposed of by the body. Analyses of the blood showed a high alkalemia, but only a slight increase of acetone bodies. The tissues contained only small quantities of acetone bodies. The fact that the base was excreted and that only small amounts of the acetone bodies were present in the tissues at autopsy indicates that the acetoacetate was burned.

With the possible exception of the fasting phlorhizinized dogs, the results appear to indicate that the dog is able to oxidize acetoacetate without intervention of carbohydrate metabolism, and that this animal is consequently not suited to the study of the relation of carbohydrate to antiketogenesis, which relation is believed to exist in man.

THE EFFECT OF ACIDOSIS ON THE SUGAR METABOLISM.

By ALFRED E. KOEHLER.

(From the Department of Medicine, Henry Ford Hospital, Detroit.)

It has been frequently observed that those physiological upsets associated with an acidosis such as anoxemia or anesthesia usually cause an increase in the fasting blood sugar.

We have noted that the production of a simple acidosis by the ingestion or intravenous injection of acids or acid-producing substances such as H_3PO_4, NH_4Cl, or $CaCl_2$ resulted in a definite increase in the fasting blood sugar of normal human subjects. A change in the venous blood reaction from approximately pH 7.40 to 7.20 was associated with an increase in the fasting blood sugar of about 30 to 40 mg. per 100 cc.

A change by the same means of similar magnitude in the blood pH of several diabetic patients studied under the same dietetic conditions resulted in a much greater and more variable increase in the fasting blood sugar. It was also noted that the blood pH of some diabetic patients was very much more easily shifted in the acid direction by even small amounts of acid intake.

In the several diabetic patients with acidosis (blood pH 6.80 to 7.20) huge doses of insulin (100 to 300 units in 12 hours) had no marked effect, but when alkali in the form of 3 per cent $NaHCO_3$ solution was administered intravenously in just sufficient quantities to return the blood reaction to normal, the blood sugar decreased very rapidly.

The depressing effect of acidosis upon the sugar metabolism may be explained at least in part by a variation of Murlin's experiments on the inactivation of insulin by glucose. We have noted that insulin (Lilly) incubated at body temperature with a 0.25 per cent glucose solution was only mildly inactivated at a pH of 7.40 and markedly at 6.80. It is thus possible that the acidotic depression of the sugar metabolism is related to an acceleration of the inactivation of insulin by glucose due to increased H ion concentration, especially when there is an overproduction or mobilization of the latter.

STUDIES ON CAPILLARY BLOOD SUGAR.

By H. V. GIBSON.

(*From the Psychiatric Institute, University of Wisconsin, Madison.*)

Since previous investigations of blood sugar in the human have been made chiefly on venous blood, more information concerning arterial sugar phenomena is pertinent. Foster has shown that capillary blood approximates arterial blood in its sugar content. A suitable micro method for blood sugar was devised, using 0.1 cc. of blood and a modification of the Folin-Wu colorimetric method. The maximum error of the method is 3 per cent.

Studies were made on capillary blood following oral administration of glucose and the data plotted. Females during the child-bearing period were found to have different shaped sugar curves from those of males, and of females past menopause. Pregnant females were found to have this variation exaggerated. A peculiar type of diabetes in pregnancy was found to have a normal sugar curve while showing acetone and glucose in the urine. This condition disappeared following delivery. A fairly constant notching occurred in the ascending limb of the curve, probably due to beginning storage in the liver. Venous sugar was found

to exceed arterial sugar in the first part of the curve of a diabetic. Hypopituitarism was associated with a low arterial concentration, demonstrating that in this type of disorder, the marked storage occurs before the sugar reaches the peripheral circulation. Acromegalic patients showed abnormally high sugar curves in the capillary blood.

A QUANTITATIVE ANALYSIS OF THE FATE OF SUGAR IN THE ANIMAL BODY.

BY CARL F. CORI AND GERTY T. CORI.

(*From the State Institute for the Study of Malignant Disease, Buffalo.*)

An attempt has been made to follow the fate of sugar in the animal body on a quantitative basis. The experiments were carried out as follows. Male rats of 120 to 150 gm. body weight were fasted for 48 hours. The urine of the last 24 hours was collected quantitatively for N determinations. The fasting metabolism of the rats was then determined for a period of 2 to 3 hours in a metabolism apparatus of the Haldane type. The temperature of the animal chamber varied between 27 and 28°C. The rats were then fed a known amount of glucose solution by stomach tube and immediately replaced in the metabolism apparatus. After 4 hours the rats were killed and the intestinal tract quickly removed. The carcass was frozen with compressed CO_2, minced, and introduced into boiling 60 per cent KOH for glycogen determinations. The amount of glucose remaining in the intestine was determined and subtracted from the amount of glucose fed. The difference between these two values was the amount of glucose absorbed during the metabolism period. The amount of sugar oxidized was calculated from the non-protein R.Q. From the amount of glycogen found, the amount of glycogen present in rats fasted for 48 hours had to be subtracted. Nine rats, fasted for 48 hours, contained as an average 0.116 per cent glycogen, maximum 0.132, minimum 0.098 per cent.

The amount of glucose oxidized plus the amount of glucose converted into glycogen amounted to 89.7 per cent of the total amount of glucose absorbed (average of ten experiments). The ratio, glucose oxidized: glycogen formed was of the order 1:1.3.

In a second series of experiments insulin was injected simultaneously with the sugar feeding. 90.9 per cent of the absorbed sugar was accounted for (average of nine experiments). The ratio, glucose oxidized: glycogen formed was of the order 1:0.9.

These experiments disprove the current theory that sugar is converted into an unknown substance during insulin action. Definite evidence was obtained that insulin increases the sugar oxidation.

THE METABOLISM OF GALACTOSE.

III. THE INFLUENCE ON THE TOLERANCE OF DISTURBED ENDOCRINE FUNCTION.

By ALLAN WINTER ROWE.

(*From the Department of Chemistry, Evans Memorial, Boston.*)

Galactose is a sugar not foreign to the human economy and yet of so low a tolerance that test meals are never productive of gastrointestinal disorders. The sugar is given to the fasting patient and urine collected at 2 hour intervals, both before (control) and after the test meal. Tolerance level is defined as the smallest amount of galactose which will produce a brief appearance of sugar in detectable amount in the urine. The higher normal tolerance of the female (40 gm.) over that of the male (30 gm.) is intrinsic.

The present report deals with the application of this test to nearly 400 subjects, most of whom had a demonstrable endocrine disorder. The results lead to the following conclusions.

The posterior lobe of the pituitary exercises the most profound influence on the tolerance; the thyroid a slight influence only but in the same sense as the pituitary, *i.e.* lowered function determines increased tolerance and *vice versa*; the testicle is without influence, while ovarian failure may cause a fall of 50 per cent in the tolerance level. Pluriglandular cases as observed exist only as the result of surgery and show the algebraic sum of the two influences involved. Adrenal failures apparently show a downward tendency. In the group of non-endocrine cases, lesions of the central nervous system, syphilis, malignancy, and anemia seemingly operate to lower the tolerance.

FURTHER OBSERVATIONS ON THE RELATION OF VITAMIN E TO REPRODUCTION IN RATS ON SYNTHETIC AND MILK DIETS.

By H. A. MATTILL and M. M. CLAYTON.

(*From the Department of Vital Economics, University of Rochester, Rochester, New York.*)

Sterility produced in rats by synthetic rations devoid of vitamin E is identical with that which we observed in animals on lard-containing milk rations. On various modifications of a basal ration containing 18 per cent of casein with starch, lard, salt, yeast, and cod liver oil, females showed resorptions. These ceased when the males were 4 to 5 months of age due to the degeneration of the testes. Externally the animals appeared normal. The inclusion of wheat germ oil in the basal ration temporarily accelerated growth in both sexes but restored fertility only to the females. This restoration was more rapid and the number of young born larger with increasing quantities of the oil (1 to 4 per cent), but lactation was very inadequate even in animals receiving a 5 per cent addition from the age of weaning. The addition of 7.5 per cent of wheat germ (less than 1 per cent of oil) not only restored fertility but secured fair lactation. None of the male animals, with a single exception, was restored by wheat germ or the oil unless these were supplied before the age of 4 to 5 months. Functional tests only can differentiate between prophylaxis and cure. Lard rendered at low temperature was not superior to commercial lard in the basal ration, nor did radiation of the animals or of the basal ration prevent sterility.

THE RÔLE OF VITAMIN E IN LACTATION.

By BARNETT SURE.

(*From the Department of Agricultural Chemistry, University of Arkansas, Fayetteville.*)

Vitamin E regulates two physiological processes. It prevents resorption of the fetus during gestation, and influences lactation after the young are born. As little as 1 mg. per animal per day of a concentrate of unsaponifiable matter from wheat oil was found sufficient to prevent sterility, but none of the normal young born were weaned. About 76 out of 100 young given to mothers to rear have been weaned on 4 mg. per day of a concentrate from wheat oil (practically free from sitosterol). Reducing the same concentrate to 2 mg. per animal per day, only 9 young out of 60 were weaned, although healthy, normal litters were born. Oxidation by aeration of acetone and ethereal extracts of wheat oil for 24 hours at 90 and 110°C. showed no deleterious effect on fertility but produced considerable mortality of the young during lactation. It is perfectly evident from work so far completed that the reproductive dietary complex, termed vitamin E, contains a hitherto unrecognized lactation factor. Whether or not it is necessary to postulate the existence of a specific vitamin for lactation will become more apparent later.

THE CHEMISTRY OF VITAMIN A.

By J. C. DRUMMOND, H. J. CHANNON, and K. H. COWARD.

(*From the Biochemical Department, University College, London, England.*)

The unsaponifiable matter of cod liver oil was extracted, dissolved in methyl alcohol, and the greater part of the cholesterol removed by crystallisation at −10°C. The rest was precipitated out with digitonin. Apparently none of the vitamin was removed by this process. From material obtained in this way it was determined that the vitamin is volatile in steam and that it distils chiefly at a temperature of 180–220°C. under 2 to 3 mm. pressure. It contains only carbon, hydrogen, and oxygen; no nitrogen or iodine. Spinacene, which is not the vitamin, may be identified in this fraction and estimated roughly by precipitating as the hydrochloride or duodecabromide. Allowing for this, the spinacene-free residue containing the vitamin is found to have an iodine value of about 103, an acetyl value of about 215, and a molecular weight of about 300. These figures would suggest that the other main constituent or constituents of the active fractions might be one or more unsaturated alcohols containing one hydroxyl group and one ethylene linkage. Such of the higher alcohols as batyl alcohol, oleyl alcohol, and selachyl alcohol occurring in certain liver oils are without physiological activity. Phytol is also inactive. It is wholly uncertain whether the higher alcohol indicated in the active fraction is the vitamin or whether the vitamin is some other substance occurring in still smaller quantity. It has no aldehydic properties and is not destroyed by acetylation or benzoylation.

A STUDY OF THE VITAMIN A AND B CONTENT OF MIXED HUMAN MILK.

By ICIE G. MACY, JULIA OUTHOUSE, M. LOUISA LONG, and B. RAYMOND HOOBLER.

(From the Nutrition Research Laboratories of the Merrill-Palmer School, the Children's Hospital of Michigan, and the Woman's Hospital and Infant's Home, Detroit.)

From biological experiments it has been shown that 10 cc. of human milk daily supplied sufficient vitamin A,[12] whereas a similar quantity may produce fair growth[13] or it may be entirely inadequate for the vitamin B[12] requirement of the rat. Similarly, 20 cc. failed to protect fowls against polyneuritis.[14] Since few data on the vitamin content of breast milk are available, the experimental results obtained in a preliminary investigation are presented.

A daily composite sample of milk from ten to sixteen wet nurses was fed to growing rats receiving a purified basal ration made adequate in respect to all the known dietary essentials with the exception of the vitamin under investigation; this was furnished by the milk. The vitamins and milk were fed fresh daily in separate dishes apart from the dried food mixture. In the vitamin B studies all animals were kept on raised screens. Individual food records were ascertained; daily vaginal smear examinations made; whenever possible, the ability of the female to rear normal young was tested.

In no instance was it possible to secure growth with quantities less than 12 cc. of milk daily as the only source of vitamin B, and it was not until 20 cc. were given that growth approximated the normal; levels of 25 and 30 cc. daily stimulated simultaneously growth and consumption of the basal ration. There was a tendency towards delayed sexual maturity in the females on milk levels below 18 cc.; in some animals normal ovulation took place when a minimum of 20 cc. of breast milk were consumed, whereas others have required 25 cc. and 30 cc.

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[12] Kennedy, C., Palmer, L. S., and Schlutz, F. W., *Tr. Am. Pediat. Soc.*, 1923, xxxv, 26.

[13] Osborne, T. B., and Mendel, L. B., *Carnegie Institution of Washington, Year Bk. No. 19*, 1920, 389; *Biochem. J.*, 1922, xvii, 363.

[14] Gibson, R. B., *Philippine J. Sc., Sect. B*, 1913, viii, 475.

Experiments indicate that 1 and 1½ cc. of human milk as the only source of vitamin A are inadequate over a long period of time. If the animal body is permitted to retain its vitamin A store, the rat has the ability to attain normal growth on 2 cc., but when the store is first exhausted this quantity is not always sufficient for normal bodily functions. There has been no apparent superiority of 15 cc. over 2 cc., females on a 5 cc. level as well as those on 10 and 15 cc. levels have produced and reared normal young. Reproduction on lower levels of milk for vitamin A as well as for vitamin B is being studied. This preliminary report deals with but one phase of a series of studies on the chemical and biological aspects of human milk production which are now in progress in our laboratories.

THE ANTIRACHITIC PROPERTIES OF CERTAIN LIPOIDS.

By ELIZABETH M. KOCH, MEYER H. CAHAN, AND REUBEN G. GUSTAVSON.

(From the Department of Pharmacology, University of Illinois, Chicago, and the Department of Physiological Chemistry, University of Chicago, Chicago.)

In October, 1924, an investigation of the antirachitic properties of certain lipoids was begun. In confirmation of the work reported by Hess and Steenbock, cholesterol, radiated dry, was found to be active in preventing rickets in rats fed the McCollum diet, No. 3143. Purified oleic and palmitic acids radiated and added to the food caused a remarkable acceleration in growth followed by severe rickets. Radiated lecithin from brain was inactive except in large amounts. This protective action was probably due to the traces of cholesterol present. Radiated tyrosine also proved of no value.

In the attempt to learn something further of the changes occurring on radiation, a dry sample of cholesterol purified by repeated crystallization from acetone was radiated 1½ hours and extracted with liquid ammonia by the method of Gustavson. A small amount of brown resinous material was separated. Non-radiated cholesterol extracted in a similar manner was completely insoluble.

The brown, liquid ammonia-soluble residue was incorporated with a weighed portion of food and fed in amounts to give daily doses of 0.25 mg. of residue to each of two rats. A remarkable improvement in the growth and general condition of these rats followed the administration of this material. A second preparation which stood a month after extraction before incorporating with food was entirely inactive in 0.3 mg. doses.

The non-saponifiable fraction of cod liver oil was extracted with liquid ammonia and again a brown, gummy residue was obtained. This fed in daily doses of 2 mg. prevented rickets entirely.

A RELATION BETWEEN APPETITE AND THE ENERGY FACTOR IN NUTRITION: A PRACTICAL CONSIDERATION.

By GEORGE R. COWGILL.

(*From the Department of Physiological Chemistry, Yale University, New Haven.*)

Our feeding experiments on dogs have shown that when a "complete" diet is fed, the animal eats all food offered and gains in weight until a certain maximum is reached; thereafter the dog eats such amounts of food daily as are necessary to maintain the body weight between rather narrow limits. When various diets differing markedly in energy content are fed, it is found that while the animal varies its daily food intake considerably, it maintains over any period of a week or more a caloric intake that is practically constant. In other words the animal *eats calories.* This idea in itself is not new, Rubner[15] having voiced his belief in it; many other students of nutrition also have assumed it to be true.

In metabolism experiments it is frequently desired to maintain a constant daily food intake. This involves among other things the administration of amounts of vitamin B sufficient to maintain the appetite.[16] As the writer has previously pointed out, metabolism animals may fail to eat all of the food offered, not because of any vitamin or other deficiency but because too many calories are being fed. Such an animal owes its *apparent* loss of appetite to an adjustment of its energy intake to its energy needs. A control of the energy factor in relation to appetite is obviously desirable.

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[15] Rubner, M., Die Gesetze des Energieverbrauches bei Ernährung, Leipsic, 1902, p. lxxxiii; quoted by Lusk, G., The elements of the science of nutrition, Philadelphia and London, 2nd edition, 1909, 218.

[16] Cowgill, G. R., *J. Biol. Chem.*, 1923, lvi, 725.

On the basis of numerous data at hand from feeding experiments carried out during the past 3 years and from the feeding of laboratory animals by students in this laboratory, it has become possible to construct tables of energy intake suitable for dogs fed according to our method described elsewhere[16] and compatible with the maintenance of appetite and excellent physiological condition while living in metabolism cages. In other words a control of the energy factor in relation to appetite for these experimental conditions has been worked out.

STUDIES IN METHYLATION.*

By N. JEAN NOVELLO, BENJAMIN HARROW, and C. P. SHERWIN.

(*From the Research Laboratory of Physiological Chemistry, Fordham University, New York.*)

In an attempt to study detoxication by methylation *in vivo*, the following substances were fed to dogs and rabbits: imidazole, pyridine, quinoline, piperidine, and quinaldine. Imidazole was largely destroyed in the body, but was not detoxicated by methylation. It yielded ammonia, some urea, but considerable allantoin and uric acid. Pyridine was qualitatively but not quantitatively methylated in the organism of the dog. Piperidine proved extremely toxic, but was to some degree excreted as an ethereal sulfate. The interesting reactions were encountered with quinoline, which was oxidized through the benzene ring yielding a pyridine derivative. Quinaldine on the contrary was oxidized through the pyridine ring, yielding some acetylaminobenzoic acid. Imidazole lactic acid, imidazole pyruvic acid, and imidazole acrylic acid were fed to rats on a histidine-free diet. As reported by W. C. Rose, we also found histidine to be an essential amino acid. Imidazole pyruvic acid is able to some extent to replace histidine in the diet; but imidazole lactic acid is much more efficient in the capacity, while imidazole acrylic acid is less so, but like the others seems to be catabolized in the body much the same as histidine. Imidazole alone has no value when used with a histidine-free diet.

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* Presented before the Society of Pharmacology and Experimental Therapeutics.

DESTRUCTION OF AN HYDANTOIN NUCLEUS (BETA-METHYL HYDANTOIN) IN THE ANIMAL BODY.*

By O. H. GAEBLER.

(*From the Biochemical Laboratory, State University of Iowa, Iowa City.*)

In several experiments in which 1½ to 4 gm. of beta-methyl hydantoin were administered to a dog subcutaneously, it was found that only 15 per cent of the compound was excreted unchanged, and that an equal amount appeared as methyl hydantoic acid. The increase in total nitrogen accounted for all of the hydantoin derivative administered. Creatinine and creatine values show no change when corrected for the amount of beta-methyl hydantoin and methyl hydantoic acid excreted. Allantoin is unaffected. Urea excretion, determined by urease, rises sufficiently to account for 20 per cent of the compound given. Oxalic acid output is increased 50 times. This, with results for amine nitrogen in Kjeldahl distillates, suggests that beta-methyl hydantoin is converted to methyl hydantoic acid, which is then hydrolyzed to glycollic acid and methyl urea, the former being oxidized to oxalic acid, and the latter in part converted to urea.

In the course of preparation of beta-methyl hydantoin from creatinine, experiments on the course of this reaction were carried out. These indicate that the methyl hydantoic acid formed by action of baryta on creatinine originates largely from condensation of sarcosine and urea with elimination of ammonia, rather than directly from creatine, or from creatinine by way of beta-methyl hydantoin.

Beta-methyl hydantoin gives a strong positive reaction when Rothera's nitroprusside test for acetone bodies is applied. Methyl hydantoic acid gives a negative test.

INTESTINAL ABSORPTION OF CALCIUM AND PHOSPHORUS.*

By OLAF BERGEIM.

(*From the Department of Physiology and Physiological Chemistry, University of Illinois, College of Medicine, Chicago.*)

A new and simple method is described for the study of absorption from the different parts of the gastrointestinal tract and for the determination of the ultimate utilization or digestibility of foods. The application of the method to the study of the absorption of calcium and phosphorus is discussed and data presented showing the influence of various factors on the absorption of these elements, particularly in rickets.

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* Presented before the Society of Pharmacology and Experimental Therapeutics.

THE EFFECT OF ANESTHESIA ON PHOSPHATE METABOLISM.*

By ADOLPH BOLLIGER.

(*From the Henry Ford Hospital, Detroit.*)

Phosphate metabolism in dogs, as indicated by studies on blood and urine, has been followed during and after anesthesia produced by ether, chloroform, ethylene, chloretone, urethane, and amytal. In contrast to the work of Potter, Martland, and Robison and Stehle and Bourne it is found that in most instances the anesthetics mentioned depress blood phosphates. This depression starts simultaneously with administration.

Consistent elevation of blood phosphates is coincident with asphyxia, excessive struggling during induction, forced rapid induction, and unusually long deep anesthesia.

The recovery period following anesthesia in these experiments is marked by a depression in blood phosphates to approximately the same low level regardless of the rise or fall during the anesthesia itself. During this recovery period the phosphate content of the blood drops to a level below the kidney threshold so that none is excreted in the urine for a period of an hour or more.

The two common adjuncts of general anesthesia, morphine and atropine, affect the phosphate metabolism, morphine lowering the phosphate level for a period of 1 or 2 hours and atropine producing a slight elevation. After the initial depression from morphine the phosphates rise above the normal level. When used with general anesthesia this period of elevation from the morphine neutralizes to some extent the phosphate depression of the anesthetic.

THE PROMOTER ACTION OF ADRENALIN ON PTYALIN.*

By E. W. ROCKWOOD and ANNA K. KELTCH.

(*From the Department of Toxicology, State University of Iowa, Iowa City.*)

Digestion was carried on at 38° with varying concentrations of starch and adrenalin, its rate being based upon the reducing sugar produced. The pH was kept constant. When the amount of starch was relatively large there was a marked promoter effect from the adrenalin, in general proportional to the amount of adrenalin present. This was shown by the natural adrenalin, adrenalin hydrochloride, and synthetic adrenalin. While the limits of its activity were not determined its effects were strongly marked at a dilution of over one per million.

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* Presented before the Society of Pharmacology and Experimental Therapeutics.

With a substrate of liver glycogen adrenalin has a similar effect upon ptyalin. This was not observed, when adrenalin was added to pulped liver tissue, nor with a solution of liver glycogen with pulped liver in the presence of adrenalin.

DIHYDROXYACETONE METABOLISM.*

By W. R. CAMPBELL, A. A. FLETCHER, J. HEPBURN, and J. MARKOWITZ.

(From the Departments of Medicine and Physiology, University of Toronto, Toronto, Canada.)

The triose dihydroxyacetone has been credited with being metabolized without the use of insulin and, under such circumstances, with possessing antiketogenic powers. It has been assigned a rôle as an intermediary metabolite of glucose. It has not previously been detected in the blood stream even after administration of considerable amounts.

A method of estimation of dihydroxyacetone in solution in the presence of glucose has been devised. It consists in reducing molybdenum in phosphoric acid solution and then reoxidizing with permanganate.

By this method, which detects one part in half a million, no dihydroxyacetone could be found in normal blood. In normals given 100 gm. of dihydroxyacetone by mouth the substance is absorbed and can be detected in the blood stream in considerable amounts.

A number of substances have been shown to cure insulin hypoglycemia. All, however, must be converted into glucose or mannose before they are effective. Insulin hypoglycemia in patients has been cured many times by administration of dihydroxyacetone by mouth. Improvement is somewhat slower than with glucose.

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* Presented before the Society of Pharmacology and Experimental Therapeutics.

To avoid the subjective side of the reaction and to study the intravenous administration of dihydroxyacetone in hypoglycemia a liberal overdose of insulin was given intravenously to rabbits. When convulsions appeared 1 gm. of dihydroxyacetone was injected intravenously. There was relief from the symptoms of convulsions and coma but not quite so rapidly as with glucose. The dihydroxyacetone in the blood falls; the total and true blood sugar rises; and the animal remains cured when the triose is given. The additional glucose can come from nowhere but the dihydroxyacetone administered.

In the fasted depancreatized dog dihydroxyacetone given by mouth is recovered quantitatively in the urine as glucose in the same time as if glucose itself were fed. There is no reduction in the total acetone body excretion during the day. The total blood sugar rises, the dihydroxyacetone in the blood increases, then disappears, and the true blood sugar rises parallel to the disappearance of the dihydroxyacetone.

When the glucose in a constant diet and insulin régime is replaced by an equal weight of dihydroxyacetone the glucose output of a depancreatized dog remains the same, thus showing no preferential utilization of dihydroxyacetone over glucose with the same amount of insulin. In the fasting depancreatized dog the respiratory quotient falls after administration of dihydroxyacetone the same as after the administration of glucose.

Some importance has been assigned the phosphates in carbohydrate metabolism. In normal human subjects the administration of equal quantities of dihydroxyacetone and glucose produces identical results on the phosphate curve.

The human diabetic patient behaves similarly to a partially depancreatized animal, utilizing, or not utilizing, the dihydroxyacetone according to his remaining capacity to produce insulin. Where the capacity for stimulation remains the blood sugar may fall; with severe cases, however, addition or substitution of dihydroxyacetone in the diet causes hyperglycemia and glycosuria accompanied by a respiratory quotient at the fat level, as in the fully depancreatized animal, and no improvement in the ketosis occurs. The evidence available, in our opinion, precludes any possibility of dihydroxyacetone possessing a greater clinical value than other substances, such as glycerol, which are more slowly converted into glucose than the starches. It also appears doubtful that dihydroxyacetone is in the main path of intermediary metabolism of carbohydrate in the body.

THE OPTICAL ACTIVITY OF CYSTEINE.

By JAMES C. ANDREWS.

(From the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia.)

Contrary to statements usually found in the literature, the specific rotation of cysteine prepared from *l*-cystine equals about +9.5. The literature values range from about −12 to −16. These negative values undoubtedly resulted from incomplete reduction. Complete reduction is effected either with metals (Zn, Sn, etc.) in acid solution or by cathodic electrolysis, the latter method being by far the more satisfactory. As the rotations of various samples of cystine change from −220 to 0, the rotations of the corresponding samples of cysteine change from +9.5 to 0.

THE CHOLESTEROL CONTENT OF THE CUTANEOUS EPITHELIUM OF MAN.

By H. C. ECKSTEIN and UDO J. WILE.

(From the Departments of Physiological Chemistry and of Dermatology and Syphilology, School of Medicine, University of Michigan, Ann Arbor.)

The cholesterol content of the outer layers of human skin was determined by the digitonin method and found to be very high. The percentage of cholesterol in four normal and six pathological specimens varied from 13.5 to 24 per cent of the total lipoids, but of the samples analyzed eight gave results ranging from 18.6 to 19.5 per cent. Contrasted with this, the cholesterol content in the subcutaneous fat of man was recently reported by one of us[17] to be as low as 0.24 per cent of the total lipoids.

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[17] Eckstein, H. C., *J. Biol. Chem.*, 1925. lxiv, 797.

LACTOSE ESTIMATION UNIFORM IN TECHNIQUE WITH ESTIMATION OF GLUCOSE IN BLOOD AND URINE.

By HOWARD D. HASKINS.

(From the Department of Biochemistry, Medical School, University of Oregon, Portland.)

Haskins and Holbrook have recently modified the Shaffer-Hartmann method, and have suggested a uniform technique for estimating glucose in both blood and urine, using the micro copper reagent. The present author has extended the method to include the estimation of lactose in urine and milk, using the same reagents and technique. The modification referred to has enabled Haskins to construct a table from which the percentage of sugar (glucose or lactose) in the original blood, urine, or milk is read off opposite the cc. of thiosulfate used for titration. The results by this téchnique have been proved to be accurate.

NEW HEMOGLOBINOMETERS.

By HOWARD D. HASKINS and E. E. OSGOOD.

(From the Department of Biochemistry, Medical School, University of Oregon, Portland.)

The new permanent standard prepared by Haskins for use with the Sahli apparatus has proved satisfactory and gives accurate estimations. The Osgood-Haskins standard solution for use with the Kober or Duboscq colorimeter has been shown to be absolutely permanent and to give estimations of hemoglobin varying from absolute accuracy by less than 2 per cent.

THE RATE OF FILTRATION OF SOME AQUEOUS PROTEIN SOLUTIONS.

By SAMUEL AMBERG.

With the Technical Assistance of Frances Sawyer.

(From the Department of Pediatrics, Mayo Foundation, Rochester, Minnesota.)

In order to obtain a fairly accurate method, alundum crucibles were used with a pressure of 30 mm. of mercury. The solutions of proteins tested were gelatin, caseinogen, and mucin prepared according to the Hammarsten method from submaxillary gland of cattle.

It was found that the mucin solution filtered much slower than the gelatin or casein solutions. The rate of filtration is not dependent on the viscosity for the viscosity of blood serum is much greater than that of mucin solutions which filtered slower than blood serum. The rate of filtration does not seem to have any definite relation to the surface tension of the solution as measured with the new Du Noüy apparatus. Comparisons are made with other glucoproteins.

CURVES OF THE BLOOD AND CEREBROSPINAL FLUID SUGAR FOLLOWING GLUCOSE INGESTION IN A CASE OF CEREBROSPINAL RHINORRHEA.

By R. B. GIBSON and HERMAN DULANEY.

(From the Clinical Chemistry Laboratory of the Department of Internal Medicine, State University of Iowa, Iowa City.)

Blood and cerebrospinal fluid sugar determinations were made by the Folin-Wu and Benedict methods at half hour periods over periods of 3 hours after the ingestion of 50 gm. of glucose. The cerebrospinal fluid sugar showed similar but less pronounced variations as compared with the blood sugar with a lag of 1 hour when the maximum content was obtained. Micro adaptation of the analytical procedures permitted exact timing of the collection of the fluid.

THE EFFECT OF PROTEOLYTIC ENZYMES ON THE ACTIVE PRINCIPLE OF TUBERCULIN.

By FLORENCE B. SEIBERT and ESMOND R. LONG.

AN ACCURATE METHOD OF ESTIMATING UREA IN FOLIN-WU BLOOD FILTRATES.

By E. P. CLARK and J. B. COLLIP.

A NEW METHOD FOR THE DETERMINATION OF ALLANTOIN IN URINE.

By A. A. CHRISTMAN.

SPECIFIC ABSORPTION STUDIES UPON RENNIN.

By B. E. L. FRENCH.

IN VITRO STUDIES ON AMMONIA AND UREA FORMATION BY TISSUES.

By H. C. GOLDTHORPE.

A MODIFICATION OF THE TISDALL METHOD FOR BLOOD PLASMA CALCIUM.

By W. R. TWEEDY.

THE INFLUENCE OF AN ATHLETIC TYPE OF EXERCISE UPON THE QUANTITY OF URIC ACID, CREATINE, AND CREATININE IN BLOOD AND URINE.

By WARREN C. WADE.

THE NUTRITIONAL VALUE OF CHLOROPHYLL AS RELATED TO HEMOGLOBIN FORMATION.

By CHARLES W. SAUNDERS.

THE APPLICATION OF OXIDATION-REDUCTION POTENTIALS TO THE MEASUREMENT OF OXIDASE ACTIVITY.

By MARTIN E. HANKE and J. A. TUTA.

A STUDY OF THE BLOOD INORGANIC PHOSPHATE AS VARIED BY RACHITIC AND NON-RACHITIC DIETS.

By ELIZABETH M. KOCH and MEYER H. CAHAN.

STUDIES OF THE EFFECT OF TEMPERATURE ON THE CATALASE REACTION.

By SERGIUS MORGULIS, M. BEBER, and I. RABKIN.

CHEMICAL AND PHYSIOLOGICAL EFFECTS OF IRRADIATION WITH ULTRA-VIOLET LIGHT.

By H. STEENBOCK, C. A. HOPPERT, and B. M. RIISING.

THE SPECIFIC DYNAMIC ACTION OF VARIOUS CARBOHYDRATES IN DOGS.

By IRENE SANDIFORD and HARRY J. DEUEL, Jr.

THE METABOLISM OF GALACTOSE.

IV. THE INFLUENCE OF OVARIAN ACTIVITY ON THE GALACTOSE TOLERANCE.

By A. W. ROWE.

ABSTRACT OF THE EXECUTIVE PROCEEDINGS OF THE TWENTIETH ANNUAL MEETING.

OFFICERS AND COMMITTEES FOR 1926.

OFFICERS FOR THE YEAR 1926: *President*, E. C. Kendall; *Vice-President*, E. V. McCollum; *Secretary*, F. C. Koch; *Treasurer*, G. E. Cullen; *Additional Members of the Council*, J. B. Collip, E. A. Doisy, A. P. Mathews; *Nominating Committee*, S. R. Benedict, W. R. Bloor, F. S. Hammett, V. J. Harding, R. S. Hubbard, A. B. Macallum, P. A. Shaffer, H. C. Sherman, J. B. Sumner.

EDITORIAL COMMITTEE: T. B. Osborne, W. W. Palmer, A. N. Richards (term, 1922–27); L. J. Henderson, W. Jones, G. Lusk (term, 1924–29); O. Folin (Ch.), A. Hunter, L. L. Van Slyke (term, 1926–31).

EDITORIAL BOARD: S. R. Benedict, H. D. Dakin, L. B. Mendel, D. D. Van Slyke.

NOMINATIONS FOR DIRECTORS OF THE JOURNAL OF BIOLOGICAL CHEMISTRY: S. R. Benedict, H. D. Dakin, G. Lusk, H. C. Sherman, D. D. Van Slyke.

FINANCE COMMITTEE: H. D. Dakin (Ch.), S. R. Benedict, D. D. Van Slyke.

ASSISTANT TREASURER: Irving Bank-Columbia Trust Co., New York.

COUNSEL: Mr. Lloyd N. Scott, 63 Wall St., New York.

NATIONAL RESEARCH COUNCIL REPRESENTATIVE: P. A. Shaffer (June 30, 1927).

NEW MEMBERS: J. C. Andrews, Jeanette A. Behre, A. Bodansky, M. T. Bogert, A. Chanutin, C. F. Cori, H. E. Dubin, H. C. Eckstein, T. E. Friedemann, M. E. Hanke, A. M. Hjort, W. G. Karr, Elizabeth M. Koch, G. H. W. Lucas, J. M. Luck, Icie G. Macy, Helen S. Mitchell, N. W. Rakestraw, C. S. Robinson, A. W. Rowe, Irene Sandiford, Florence B. Seibert, E. S. West, E. J. Witzemann, Ella Woods, E. J. Young.

CLINICAL MEMBERS: B. Hamilton, M. Kahn.

RESIGNATION ACCEPTED: C. S. Hudson.

DECEASED MEMBERS: F. C. Cook, John Marshall.

ATTENDANCE: Over eighty members attended the meetings.

REPORT OF THE EDITORIAL COMMITTEE.

Toward the end of the year 1925 the editorial management of The Journal of Biological Chemistry was transferred from the Rockefeller Institute to Cornell Medical College, and Dr. S. R. Benedict replaced Dr. Donald D. Van Slyke as managing editor. This change was the outcome of a decision on the part of the Rockefeller Institute authorities to retrench their publication activities.

The rapidly increasing activity in American biochemical research and the increasing tendency among foreign investigators to publish in The Journal of Biological Chemistry have raised a serious problem of how best to meet this growing need for space. More rigid scrutiny of all papers offered for publication and a more rigid insistence on conciseness of presentation is imperative. These necessary demands unfortunately will increase still further the work, already very heavy, on part of the members of the editorial board.

Economically the Journal is still self-supporting without any necessity for drawing upon the Herter Fund which now amounts to more than $74,000. Any policy of expansion which would largely increase the annual cost of publication without correspondingly increasing the income in the form of additional subscriptions, could easily absorb much more than the income from the Herter Fund. The income from this fund for the year 1925 amounted to $3,575.00. The question has been raised, and will doubtless come up for future discussion, whether all members of the American Society of Biological Chemists should not be required to subscribe to the Journal. All who can afford to subscribe are urged to do so. Up to the present the editorial committee has not seen the subscription list and therefore cannot state how many of the members of the society are supporting the Journal.

For the Editorial Committee.
[Signed] OTTO FOLIN.

ABRIDGED REPORT OF THE FINANCE COMMITTEE.

Summarized Statement of Receipts and Disbursements, Nov. 30, 1924–Dec. 1, 1925.

American Society of Biological Chemists, Inc. (Christian A. Herter Memorial Fund) in account with Irving Bank-Columbia Trust Co., New York.

Receipts.

		Disbursed.	*Received.*
1924			
Nov. 30	Balance		$3,241.89
	Income from bonds		3,575.00
	Bank interest		20.14
	Disbursements.		
1924			
Dec. 8	3000 New Orleans, Texas and Mexico Railway 5½s 1954 at 99 plus commission and accrued interest	$3,005.21	
Dec. 23	Commission for acting as custodian of securities and services rendered for one year ending December 12, 1924	50.00	
1925			
July 6	2000 New Orleans, Texas and Mexico Railway 1st 5½s at 101½ plus commission and accrued interest	2,061.11	
July 10	1000 American Smelting and Refining Co. 5s 1947 at 98⅜ plus commission and accrued interest	990.61	
Dec. 1	Balance	730.10	
		$6,837.03	$6,837.03
Dec. 1	Balance		$730.10

The Treasurer reported a balance of $208.35 on hand. The Treasurer's books were audited by Drs. Shaffer and Cullen and found to be correct. The dues for 1926 were fixed at $2.00.

The Society voted to change the time of meeting for one year. The Executive Committee of the Federation favor a meeting some time in the spring of 1927.

The Society endorsed the work of the American Association for Medical Progress, a society organized to support and defend the scientific method, medical sciences, and physiological experimentation, and voted to become a corporate member. The desirability of members of the Society becoming members of the Association was emphasized.

Dr. S. R. Benedict, the managing editor of The Journal of Biological Chemistry, in speaking of the large number of papers which were being sent to the Journal for publication asked the Society for authorization to publish one additional volume in 1926. A motion was passed that the Editorial Committee should not hesitate to publish as many volumes as they thought necessary or advisable.

D. WRIGHT WILSON,
Secretary.